FORM PTO-1390 (REV 10-95) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. §371

ATTORNEY'S DOCKET NUMBER: LAMILL 1

U.S. APPLICATION NO. (If known, see 37 CFR §1 5): 09/807502

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/US99/23860	15 OCTOBER 1999	16 OCTOBER 1998

TITLE OF INVENTION

METHODS OF MAKING PATTERNED ARRAYS OF ANALYTE-BINDING MOLECULES

APPLICANT(S) FOR DO/EO/US

MILLSTEIN, Larry S.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ■ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. §371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. §371.
3. ☐ This express request to begin national examination procedures (35 U.S.C. §371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. §371(b) and PCT Articles 22 and 39(1).
4. ■ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ■ A copy of the International Application as filed (35 U.S.C. §371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ■ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. §371(c)(2)).
7. ■ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. §371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ■ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. §371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. §371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. §371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. §§1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. §§3.28 and 3.31 is included.
13. ■ A FIRST preliminary amendment.
    ☐ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

U.S. APPLICATION NO. (if known, see 37 CFR §1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/807502	PCT/US99/23860	LAMILL 1

17. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 CFR §1.492 (a) (1) - (5)):	
Search Report has been prepared by the EPO or JPO..... $860.00	
International preliminary examination fee paid to USPTO (37 CFR §1.482)..... $690.00	
No international preliminary examination fee paid to USPTO (37 CFR §1.482) but international search fee paid to USPTO (37 CFR §1.445(a)(2))..... $710.00	
Neither international preliminary examination fee (37 CFR §1.482) nor international search fee (37 CFR §1.445(a)(2)) paid to USPTO..... $1000.00	
International preliminary examination fee paid to USPTO (37 CFR §1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)..... $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$860.00
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(e)).	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	21 - 20 =	1	x $ 18.00	$18.00
Independent claims	2 - 3 =	0	x $ 80.00	$0.00
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $ 270.00	

TOTAL OF ABOVE CALCULATIONS =	$878.00
Reduction of 1/2 for filing by small entity, if applicable. Applicant qualifies.	$439.00
SUBTOTAL =	$439.00
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. §1.492(f)).	
TOTAL NATIONAL FEE =	$439.00
Fee for recording the enclosed assignment (37 C.F.R. §1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. §§3.28, 3.31). $40.00 per property.	
TOTAL FEES ENCLOSED =	$439.00
Amount to be refunded:	
charged:	

a. ■ A check in the amount of $439.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 13-3402 in the amount of $ _____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ■ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 13-3402. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 C.F.R. §§1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. §1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO: Customer Number 23,599

23599
PATENT TRADEMARK OFFICE

SIGNATURE

Richard J. Traverso
NAME

30,595
REGISTRATION NUMBER

Filed: 16 APRIL 2001

RJT:kms

IN THE UNITED STATES DESIGNATED/ELECTED OFFICE

International Application No.	:	PCT/US99/23860
International Filing Date	:	15 OCTOBER 1999
Priority Date(s) Claimed	:	16 OCTOBER 1998
Applicant(s) (DO/EO/US)	:	MILLSTEIN, Larry S.

Title: METHODS FOR MAKING PATTERNED ARRAYS OF ANALYTE-BINDING MOLECULES

PRELIMINARY AMENDMENT

Commissioner for Patents
Washington, D.C. 20231

SIR:

Prior to calculating the national fee, and prior to examination in the National Phase of the above-identified International application, please amend as follows:

IN THE CLAIMS:

11. (Amended) A method of claim 1, wherein the analyte-determining molecule is an antibody, an oligonucleotide, a protein-nucleic acid, an aptamer, a ribozyme, a nucleic acid, a peptide, a nucleic acid binding-polyamide, a polysaccharide, a glycoprotein, a lipid, a lectin, a receptor polypeptide, a ligand, an antigen, a fusion protein, a hapten, or a chelating agent.

12. (Amended) A method of claim 1, wherein the analyte-determining molecule is a polyclonal antibody, a monoclonal antibody, a Fab fragment, a single-chain antibody, or a disulfide Fab fragment.

13. (Amended) A method of claim 1, further comprising analyte determination.

14. (Amended) A method of claim 1, further comprising utilizing the device to determine the presence of analytes which comprises: an immunoassay, a hybridization assay, a ligand binding assay, a receptor binding assay, or an affinity binding assay.

15. (Amended) A method of claim 1, wherein the analyte-detection is by radiation, chemoluminescence, phosphophorescence, fluorescence, or energy emission.

REMARKS

The purpose of this Preliminary Amendment is to eliminate multiple dependent claims in order to avoid the additional fee. Applicants reserve the right to reintroduce claims to canceled combined subject matter.

Respectfully submitted,

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RJT:jmm

METHODS OF MAKING PATTERNED ARRAYS OF ANALYTE-BINDING MOLECULES

This application is related to the following applications which are incorporated by reference in their entirety and of which priority is claimed, as noted below, to the extent permissible in any Authority wherein the application is filed:

US Provisional Application No. 60/062,203 filed on 16 October 1997, the contents of which are herein incorporated by reference in their entirety;

US Provisional Application No. 60/104,643 filed on 16 October 1998, the contents of which are herein incorporated by reference in their entirety and of which the present application claims priority;

PCT/US98/21860 filed on 16 October 1998, which is a continuation in part of aforementioned US Provisional Application No. 60/062,203, and published as International Publication No. WO 99/19711, the contents of which are herein incorporated by reference in their entirety and of which the present application claims priority;

US Provisional Application No. 60/104,642 filed on 16 October 1998 the contents of which are herein incorporated by reference in their entirety and of which the present application claims priority.

BACKGROUND OF THE INVENTION

Arrays are important in many technologies, and methods to make arrays precisely, efficiently and economically are of widespread importance. Recently, the value of arrays with small dimensions has been recognized and interest is high in finding methods to produce a wide variety of small scale arrays commercially.

There has become an increasing demand for the application of array technology to chemical, biochemical, biological, genetic, etc., assays. The use of arrays to carry out such assays has many wide applications in the healthcare, environment, and information technology industries. Discrete assay devices for a wide variety of physical, chemical, biochemical and biological attributes have

become commonplace, both in industrial and consumer applications. Among familiar devices of this type are medical test devices such as "dip sticks" that measure chorionic gonadotropin in over-the-counter pregnancy test kits, and autoanalysers that carry out clinical diagnostic chemistry testing. The use of such devices is expanding rapidly as it becomes possible to monitor an increasing number of properties and substances by highly reliable and accurate tests.

Most devices currently in use are directed to a single discrete test, such as a single assay for a particular compound. Even where a large number of assays is performed on a large number of samples, current methods typically proceed by dividing each sample into separate portions for each test and performing the tests separately. This is true even in some highly sophisticated applications. For instance, blood bank autoanalysers generally split each sample into small aliquots that are analyzed separately to determine each measured property. The same often is true of testing urine for drugs, for instance. This "divide and conquer" approach can be efficient and cost effective; but, it is not necessarily the best way to carry out very large numbers of tests on a large number of samples. The divide and conquer strategy diminishes the sample available to each assay in direct proportion to the number of tests that are performed. Hence, it is disadvantageous for tests that require relatively large sample volumes, such as tests for HIV viral load. Furthermore, the divide and conquer strategy requires separate analysis channels for each test. Complexity thus increases directly in proportion to the number of tests performed. In sum, the divide and conquer approach disadvantageously limits the test-effective sample amount and incurs additive sample manipulation, fabrication and apparatus costs that become increasingly onerous as the number of tests and the number of samples increases.

Disadvantages of divide and conquer strategies are overcome by arrays. For instance, several companies have demonstrated devices for DNA-based diagnosis that have a thousand or more different sequence-specific probes on a single assay surface where each one can be individually addressed. Clearly, it would be difficult and impractical to divide each sample into thousands of

aliquots to test individually against all these probes, as required by divide and conquer strategies. Instead, all of these devices separate the sequence specific probes into discrete locations in a defined pattern on a surface and expose all probes to the sample at the same time. Results are determined by detecting where the sample hybridizes to the array. All the probes access the entire sample, avoiding the dilution-by-aliquoting effect of divide and conquer strategies. And, the sample is hybridized to all the probes in a single reaction, greatly simplifying the process and reducing its cost. All-at-once approaches using arrays clearly are more effective than divide and conquer strategies for carrying out a large number of assays on only a limited sample. The approach has even more impressive benefits for carrying out a large number of assays on a large number of samples. Widespread availability of arrays thus would be of great benefit in this regard.

Unfortunately it has been possible to make arrays for such applications only by two relatively inefficient and difficult methods: spotting and positional solid phase synthesis. A variety of devices have been used to make arrays by spotting materials onto a surface, including contact spotters and ink jet-like spotters. A contact spotter has been designed and employed by Brown and colleagues at Stanford University to make DNA probe arrays on various surfaces, typically for profiling expression of many genes at once. (See, for instance, the Brown web page at http://cmgm.stanford.edu/pbrown.). This spotter, and other spotter designs, also have been used by many genomics and expression profiling companies, including but not limited to Incyte, Incyte/Synteni, Hyseq and Millennium. (See the web pages and literature of the companies.) For example, scientists at Hyseq reported spotting 8,192 oligonucleotide probes for sequencing-by-hybridization onto a flat surface using a contact spotter of this type. (See Drmanac *et al.*, *Nature Biotechnology* 16: 54-58 (1998) which is herein incorporated by reference in its entirety.) In an example of arraying using ink jet-like devices, oligonucleotide probes for a hybridization assay were dispensed by a micro-ink jet spotter directly onto the surface of a CCD. (See Eggers *et al.*, *BioTechniques* 17: 516-524 (1994) which is herein incorporated by reference in its entirety). Finally, oligonucleotide

probes have been spotted onto flow-through chips using both contact spotting and ink jet methods, as described by Beattie and colleagues and developed by Gene Logic. (See, for instance, Beattie *et al.,* WO 95/11755 which is herein incorporated by reference in its entirety.)

The spotting approach requires machinery capable of flawlessly depositing precise volumes of material at precise locations on a substrate, repeatedly. To make 10,000 array replicates of a 10,000 member array using spotting methods requires 100,000,000 spots and, therefore, at least 100,000,000 spotting operations. Accuracy and precision are very difficult to maintain over such a large number of operations, needless to say, and this can limit the use of spotting approaches, particularly for large scale production of complex arrays. In addition, spot size probably cannot be reduced below about 100 microns in diameter in practical spotting devices. If so, spotted arrays will be limited to densities of less than 10,000 assays per square centimeter and will not be suitable for many applications.

A second approach that has been used to make assay arrays involves solid phase synthesis on a surface controlled by photo lithographic techniques. Using this type of approach, the leaders in this field, Affymax and Affymetrix, have used light-addressable peptide and oligonucleotide solid phase synthesis chemistries to build up checkerboard-like arrays of short peptides or oligonucleotides. Affymax scientists initially reported synthesis of an array of 1,024 peptides and since then they have reported much larger arrays. (See, for instance, Fodor *et al.*, *Science* 251: 767-773 (1991), Pirrung *et al.*, WO 90/15070 and Pirrung *et al.*, US patent No. 5,143,854 issued 1 September 1992, which are herein incorporated by reference in their entirety.) Affymetrix scientists have reported a variety of oligonucleotide arrays. (For early results see Pease *et al.*, *PNAS* 91 5022-5026 (1994) which is incorporated by reference herein in its entirety.) One set of four arrays produced by Affymetrix included 20 pairs of 25-mer oligonucleotide probes for all 6,200 genes predicted by analysis of a complete yeast genomic sequence. (See Wodicka *et al.*, *Nature Biotechnology* 15: 1359-1367 (1997) which is incorporated by reference herein in its entirety). The arrays included over 250,000 oligonucleotides and were used, initially, to

profile expression of all yeast genes all-at-once in single hybridization experiments. The same arrays also have been used to analyze genetic selections in yeast. (See Cho *et al.*, *PNAS* 95: 3752-3757 (1998) which is incorporated by reference herein in its entirety). Smaller scale arrays of this type have been made for profiling expression of genes of other organisms, such as *E. coli* and humans. (See, for instance, Saizieu *et al.*, *Nature Biotechnology* 16: 45-48 (1998) and Lockhart *et al.*, *Nature Biotechnology* 14: 1675-1996 (1996), which are herein incorporated by reference in their entirety.) Arrays like these are used intensively in drug discovery programs to identify disease and therapy associated changes in gene expression and to identify promising targets for drug discovery and development. Affymetrix scientists also have reported oligonucleotide arrays useful for SNP discovery and screening. These include arrays for discovering human SNPs and arrays for sequencing human mitochondrial DNA. (See, for instance, Wang *et al.*, *Science* 280: 1077-1082 (1998) and Chee *et al.*, *Science* 274: 610-614 (1996).) The array for mitochondrial sequencing contained 135,000 oligonucleotide probes able to interrogate and in most cases determine the complete sequence of human mitochondrial DNA in a sample, in a single experiment. Arrays of this type are particularly useful for SNP mapping and for pharmacogenomics studies. Both types of arrays demonstrate the power and potential of arrays for all-at-once determinations on a large scale.

Another array-making approach involving combinatorial synthesis of oligonucleotides on a surface was developed by Southern. (See, for instance, Milner *et al.*, *Nature Biotechnology* 15: 537-541 (1997) which is incorporated by reference herein in its entirety). In this approach, an annular mechanism delivers reagents to selected areas of a surface in a series of addition reactions. The particular overlap of reagent exposures at various locations on the surface defines the array members.

The synthesis approach has disadvantages, however, despite the aforementioned success. First, assay diversity in this approach depends on combinatorial build-up chemistries. Because of this it is limited to combinatorial polymers, such as nucleic acids and peptides, and, it generally requires knowing

the sequences prior to the synthesis. Thus, the approach is not useful for complex molecules that cannot be synthesized and it is not effective without sequence information to guide synthesis. It does not lend itself to mixed arrays containing different kinds of immobilized reagents. The approach relies on reiteration of fairly complex steps and typically provides a low yield of array members in a high background of side products. Array members are formed *in situ* and cannot be processed, purified or assayed before use. The approach depends on high precision and reproducibility of complex synthesis steps, as well as uniformity of yield across a large number of differing products. Photo lithographic implementations require complex, high precision machinery and extremely high levels of skill, equipment and investment as does semiconductor manufacturing, from which it is partly derived. Photo lithographic approaches, thus, are as expensive as integrated circuit manufacturing, prohibitively so for many applications.

The array approach also is being applied to the discovery of new and useful inorganic materials, an approach spearheaded by Symyx, Inc. For instance, Symyx scientists combined thin film deposition and physical masking techniques adapted from the semiconductor fabrication industry to synthesize arrays (spatially addressable libraries) of solid state materials to screen for properties of interest. Arrays of inorganic compounds have been fabricated by this approach and screened for superconducting properties. These arrays contained up to 10,000 samples per square inch. Sample areas were squares as small as 200 micrometers on each side. (See Xiang *et al.*, *Science* 268: 1738-1740 (1995) which is herein incorporated by reference in its entirety.) Similar arrays made by this approach were screened for large magnetoresistance. (See Briceno *et al.*, *Science* 270: 273-275 (1995) which is herein incorporated by reference in its entirety.) More recently, a rare-earth phosphor of useful properties was discovered by combinatorial synthesis and parallel screening techniques using this approach. (See Danielson *et al.*, *Science* 279: 837-839 (1998), which is herein incorporated by reference in its entirety). In this example, approximately 25,000 different compositions were defined on a surface by depositing constant or varying thicknesses of 5 oxides

and 10 elements. The array was screened for UV photo luminescence. This method also relies on combinatorial build up of array members *in situ* (in the array) and, thus, it is not possible to assess quality of array members or to purify them before use. The method also requires resynthesis of all array members in each array. Therefore, like the synthesis approaches described above, it requires a very high level of precision and reproducibility. And the necessity to build up the array members individually in each array is more and more onerous as the number of array replicates increases, making it increasingly less practical as the number of arrays gets larger.

Further information about arrays, particularly micro-arrays of DNAs, is provided in *Nature Genetics* 21 (Supplement: THE CHIPPING FORECAST): 1-60 (Jan 1999) which is incorporated herein by reference in its entirety in parts pertinent to arrays and related topics.

Thus, while the power of arrays is proven and it is clear that they will be breakthrough tools in many areas of research and development, existing techniques for making arrays both limit the types of array that can be made, the efficiency and economy with which they can be made, and the quality of the arrays and the ways in which they can be used. Clearly, all of the available ways of making arrays, and the arrays made by these methods, have undesirable limitations. None is suited for all applications and ways of making suitable arrays have not been developed for all applications.

Accordingly, there exists a need for arrays, for ways to make arrays, and for ways to use arrays that overcome these limitations. There is a need, therefore, for better methods and devices for making arrays, for improved arrays, and for improved methods and devices for using arrays. Thus, for example, there is a need for better methods and devices for making arrays, for improved arrays, for improved methods and devices for using arrays for determining physical, chemical and biochemical properties of samples, particularly for detecting and quantifying analytes in samples, such as molecular, macromolecular and cellular analytes in chemical, biological, veterinary, clinical, medical, forensic, agricultural, environmental, food, consumer, industrial and military samples, to mention just a few examples.

In sum, while the power of arrays is clear, current array-related technology has many shortcomings and limitations and better methods and devices for making arrays, improved arrays and improved methods and devices for using arrays are needed.

BRIEF SUMMARY

It is therefore an object of the present invention to provide in certain preferred embodiments, among other things, novel and improved methods and devices for making arrays, novel and improved arrays, and novel and improved methods and devices for using arrays.

It is among particular objects of the invention in this regard to provide methods for forming arrays by iterating a cycle in which a plurality of array members are bound to a plurality of segments of a substrate or device for forming an array thereon. In a further aspect in this regard, preferred embodiments of the invention array members are bound to and/or on and/or within segments of one than one surface of a substrate or device. In certain preferred embodiments in this regard array members are bound to one surface of a substrate or device. In further preferred embodiments in this regard array members are bound to a smooth surface.

In certain preferred embodiments in this regard array members in temporally adjacent cycles are bound to and/or in and/or within segments that overlap or are adjacent to one another. In this regard, adjacent segments and/or the array members in and/or on and/or within the segments may be overlapping, non-overlapping but with edge to edge contact or non-overlapping and not in contact. Non-overlapping segments are particularly preferred in certain preferred embodiments. In certain highly preferred embodiments in this regard array members bound in and/or to and/or within non-overlapping adjacent segments of the substrate or device.

In a further aspect of the invention in this regard in certain particularly preferred embodiments in each cycle a plurality of array members is introduced into a corresponding plurality of channels in an array forming substrate or device, one or more introduced array members are bound to and/or in and/or

within a segment of the corresponding channel and an array is formed by the array members bound to and/or in and/or within segments in the plurality of channels. In another aspect of the invention in this regard, in certain highly preferred embodiments the corresponding segments of different channels lie along a line. In certain highly preferred embodiments in this regard, corresponding segments lie along a line orthogonally disposed to the parallel orientation of the channels. In highly preferred embodiments in this regard relating to two dimensional characterization of arrays, the array members are bound to segments aligned orthogonally to parallel channels form a right angle array. In certain highly preferred embodiments of this type the array members form a checkerboard-like array.

In a related aspect, in certain particularly preferred embodiments the channels are non-communicating channels. In another aspect in this regard, channels in particularly preferred embodiments are parallel to one another. In certain highly particularly preferred embodiments in this regard non-communicating channels are parallel to one another. In certain preferred embodiments in this regard, the channels are dimensionally similar or the same as one another. In other preferred embodiments in this regard channels differ from one another.

In a further aspect of the invention in this regard, in certain preferred embodiments parallel non-communicating channels are grouped in banks (referred to herein generally as channel banks). In certain highly preferred embodiments in this regard the banks comprise adjacent channels. In certain particularly preferred embodiments in this regard, banks are dimensionally similar or the same as one another and comprise similar or the same number of channels. In certain especially preferred embodiments in this regard the banks are comprised exclusively of adjacent channels.

In another aspect of the invention, preferred substrates for forming arrays are comprised of two members joined together. Particularly preferred embodiments in this regard related to channeled substrates in formed of two members. In certain highly preferred embodiments in this regard, one member comprises a surface for forming an array and the other member comprises a

surface dimensioned and/or shaped to form channels when the two members are joined together. In especially preferred embodiments in this regard the array forming surface is flat. In certain particularly preferred embodiments in another regard the two members when joined form a rigid or a flexible film or ribbon. In some aspects of the invention a flexible film or ribbon is preferred.

In certain preferred embodiments of the invention in another aspect, array members are attached and/or bonded, and/or joined, and/or bound and/ or immobilized to and/or on and/or within a substrate or device on and/or in and/or within which they form an array by activating a bonding and/or joining and/or binding and/or immobilization process in segments of the substrate or device so as to bind specific array members thereto. In certain preferred embodiments in this regard a photochemical reaction is activated to bind array members. In certain embodiments in this regard a photo "cross-linking" reaction is activated. In certain other preferred embodiments a photolytic reaction is activated. In still other preferred embodiments in this regard a combination of photo-linking and photolytic reactions are activated. In certain preferred embodiments of the invention in this regard the light used for activation is visible light or UV light. In highly preferred embodiments relating to this aspect of the invention light, devices, exposures, reagents and techniques used for the photo-lithography and photo-lithographic processes for manufacturing integrated circuits are used to activate segments of substrates and devices for forming arrays and to bind array members.

In yet another aspect the invention relates to arrays of binding reagents. In particularly preferred embodiments in this regard the binding reagents bind one or more cognate analytes. In certain especially preferred embodiments in this regard the binding reagents are effective for detecting and/or quantifying cognate analytes. Preferred binding reagents include purified molecules, mixtures thereof, complexes, aggregates and or other compositions of matter effective for binding a cognate.

It is a further aspect of the invention in this regard certain preferred embodiments provide methods for making devices comprising surface having immobilized in discrete, predetermined, identifiable positions thereon a plurality

of analyte-binding molecules. It is a particular object of the invention in this regard to provide devices comprising a surface having immobilized in discrete, predetermined, identifiable positions thereon a plurality of first members of a corresponding plurality of complementary (cognate) pairs, wherein the first and second members of each complementary (cognate) pair bind one another specifically and the binding of one member of a pair to the other can be detected and/or quantified and thus utilized to detect or measure the amount of cognates in a sample for analysis. In some particularly preferred embodiments of the invention in this regard the cognates are polynucleotides having sequences complementary to one another. In certain especially preferred embodiments in this regard the polynucleotides in the array are oligonucleotides. In other preferred embodiments in this regard the cognates are an antigen and an antibody or antibody-derived antigen-binding reagent and an antigen or antigen-analog. In further preferred embodiments in this regard the binding cognates are a glycan or glycosylated molecule and a lectin that binds thereto, a receptor and a ligand, an aptamer and its target, or another biological or synthetic binding pair.

Additional aspects of the invention relate to using arrays. Among further objects of the invention in this regard is to provide devices comprising arrays and having encoded therein or thereon identifying information, preferably in discrete predetermined locations therein or thereon, preferably in computer readable form. In certain preferred embodiments in this regard the information relates to one or more of the following: individual device identifier, processing lot number or numbers, type of device, date of manufacture, expiration date, quality control information relating to functional aspects of the device or the array, information necessary to use the device or array and information supplied by the user. In particularly preferred embodiments in this regard the information on device identity is required to use the device. In certain preferred embodiments in this regard relating particularly to devices comprising arrays for detecting and/or quantifying analytes information about each assay in the device may be provided.

It is a further object of the invention to provide devices for carrying out

array making processes of the invention, such as those summarized briefly above. It also is among the objects of the invention to provide devices for using the arrays thus made. In one preferred embodiment of the invention, the device is an autoanalyser which delivers reagents. In another preferred embodiment of the invention, reagents for developing the assays are an integral part of the device.

The foregoing summary is not comprehensive of the invention in any respect. Rather, it describes very briefly certain specific objects and embodiments of the invention in order to provide an impression of the invention, *albeit* incomplete, that will facilitate a more comprehensive understanding based on reading the present disclosure as a whole, viewed in light of the knowledge of those skilled in the arts to which the invention pertains. The summary herewith presented does not portray the limits of the invention. Rather it is directed to particular embodiments aspects thereof. A full understanding of the invention is to be had only by careful consideration of the entirety of the present disclosure in light of the knowledge of those skilled in the arts to which it pertains.

BRIEF DESCRIPTION OF THE FIGURES

The figures are provided to aid understanding of the invention herein disclosed. They portray certain specific illustrative embodiments and aspects of the invention. They do not portray the invention in its entirety in any respect and they do not portray limitations of the invention.

FIGURE 1 shows a process of the invention for forming an array. The first cycle (C1) of the process starts at (S1) with several substrates (rectangles 1 2 3 . . . n at upper left. The substrates may be separate pieces or different portions of the same piece, such as a set of channels in a ribbon substrate. In the second step (S2) the substrates are exposed to the array members. The substrates may be exposed to different array members or the same ones. II step three (S3) sectors of the substrates are activated to bind array members. In the final illustrated step (S4) substrates are cleared of unbound material

leaving array elements bound to the activated sectors of each substrate. The cycle of exposure, activation, and clearing is repeated in a second (C2) and subsequent cycle (...Cq). After q cycles the process forms the n X q array illustrated at lower right.

It will be appreciated that the figure depicts but one relatively simple embodiment. For instance, the stippling does not represent the differences or complexity possible for array members exposed to the substrates. Steps other than the four depicted here may be employed (as figuratively indicated by the vertical ellipsis). The geometry of the substrates and the array elements need not be uniform or governed by right angles. Not all substrates must be filled in each cycle, nor must they all be emptied, to mention just a few aspects of the many embodiments of the invention, which generally are further described elsewhere herein.

FIGURE 2A shows several general methods for producing many arrays at the same time. Substrates (vertical lines) are organized into several identical Banks (CB1 CB2 . . . CBn), *e.g.* banks of channels in a ribbon. The substrates pass several Activating Stations (large boxes AS1 AS2 . . . ASp). Each Activating Station contains a set of Activating Lines (thin horizontal boxes AL1 AL2 . . . ALq). that operate together. A process according to Figure 1 is carried out in all the substrates at the same time using all the activating lines. All the Banks and Activating Lines operating in synchrony can form (n)(p)(q) unit arrays at the same time. For instance, 100 Banks working with 40 Activating Lines in 10 Activating Stations can form (100)(40) = 4,000 unit arrays in one complete cycle set. A single unit array (ua) is set off by the rectangle to the right of "AL1."

FIGURE 2B shows a section of the ribbon in greater detail. CB, AL and ua are as for Figure 2A. The arrow at A indicates the activation dimension. The arrow at F indicates the flow dimension. Sixteen unit arrays are pictured.

FIGURE 3A provides a schematic illustration of an activating device. Light from a source (1) is focused by a lens (2) onto a slit plate (3) to activate a region (4) on a binding surface of a flow channel and thereby bind a reagent disposed in the lumen (6) of the flow channel. (5) is the lower member of the channel, (7) is the upper member, and (6) is the lumen. (8) somewhat fancifully

depicts light scattering as it moves away from the focal point (4) through the lumen (6) and the upper member (7) of the flow channel and out of the ribbon.

FIGURE 3B depicts a moving activation device positioned at two different points along a fixed ribbon.

FIGURE 3C depicts a moving ribbon in two different positions under a fixed activation device.

FIGURE 4A illustrates from the side features of a device for manufacturing a large number of arrays using a parallelized process of the type depicted in Figures 1 and 2, and an activation instrument along the Lines presented in Figure 3. A long ribbon (2) containing the flow channels passes above an activation station containing four activating Lines (1). The ribbon is wound on spools (3 and 6) at each end of the device. The spools can pass the ribbon back and forth so that it can be activated repeatedly anywhere along its length. Thus, reagents generally need to be loaded only once into the flow channels to be immobilized at all the desired positions in the ribbon. Reagents are introduced into and removed from the ribbon through manifolds (4 and 5) at each end. The activation area is environmentally isolated by barriers (solid and dashed Lines (7)). Generally, the device would include connected quality control instruments for determining, among other things, uniformity of solutions in the flow channels and the efficiency and uniformity of binding after activation (not shown).

FIGURE 4B illustrates from the bottom the ribbon and slit plate of the device shown from the side in Figure 4A. It depicts the channel ribbon (2) passing over the four activation Lines (1). The slits that define the areas of activation are exaggerated. In some preferred embodiments, for instance, they would be around 10 to 100 micrometers wide while the ribbon would be on the order of 10 to 100 centimeters across. The ribbon would be 10,000 times wider then the slit that runs across it in these embodiments.

FIGURES 5A - 5E show a method for forming a ribbon with flow channels as follows.

FIGURE 5A shows a portion of a channel-forming film. Relative dimensions of ribs, channels thickness are not representatively proportional.

FIGURE 5B shows a film that provides a flat smooth binding surface.

FIGURE 5C show the two films bonded together to form square channels.

FIGURE 5D shows reagents (stippling) in the channels. While channels typically will be filled, they are portrayed partially filled to enhance perspective.

FIGURE 5E shows an array of reagents affixed to the binding surface, with the channel-forming film removed. Elevation of the reagents above the surface is exaggerated from typical arrays.

FIGURE 6 illustrates some features that can be incorporated in an assay area. The analyte binding reagent or other material to be immobilized is illustrated by the 15 square elements in the center of the area, arranged in a 3 X 5 grid. Each of the square elements can provide redundancy or uniquely different immobilization features, such as a graded series of concentrations of the array member, such as an analyte binding reagent. Alignments markers (triangles with cross hatch) set off the right and left sides of each row in the grid. A "start" point, such as for reading results, is marked by the horizontally striped triangle to the left square element 1. Ends of the intermediate and last rows are marked by a down-pointing triangle with vertical stripes and a diagonally striped square, respectively. The corners of the assay area are set off asymmetrically, a different marker shape in each corner. Machine-readable coding areas are indicated by one rectangular area above the grid (horizontal striping) and three below the grid (vertical striping). The elements of the grid, the alignment and other markers and the coding areas can be formed using marks during activation, much the same as photo-lithographic processes.

FIGURE 7 illustrates assembly of chips into sub-assemblies and modules. The top of the figure shows a chip with 400 spots. The middle of the figure shows five 400-spot chips totaling 2,000 spots in a single sub-assembly. The bottom of the figure shows six sub-assemblies containing 30 chips and 12,000 spots in a single module. The figure illustrates the build up of a large device from smaller units, which provides multiple ways to incorporate a given spot into a device, and multiple chances to correct a faulty spot in any one chip or set of chips.

FIGURE 8A illustrates from the side a device for contacting a chip with samples and reagents for analysis. (1) is a chip module. (2) is a sample delivery port. (3) is a reagent delivery port. (4) is a reagent exit port. (5) is a channel for sample and reagent, which runs from the sample and reagent entry ports directly over the chip module to the reagent exit port. (6) is three areas for human and/or machine readable information on the device.

FIGURE 8B illustrates from the side the device shown in Figure 8A. The chip module (1), sample loading septum (2), reagent delivery septum (3), reagent exit septum (4), and reagent channel can be seen particularly clearly in this view.

FIGURE 8C shows a side view of the device joined to reagent delivery manifold. (7) indicates the direction of sample loading into the sample loading port, which is covered by a septum (dark rectangle (2)). (8) is a reagent delivery manifold with reagent delivery port (9) and reagent removal port 10. O-ring gaskets (black circles) provide a fluid-tight seal between the device and the manifold. Disposable "needles" in (9) and (10) puncture the septa (3) and (4) to isolate the delivery channels of the manifold from the channel and other parts of the device.

GENERAL DESCRIPTION

The present invention relates generally to methods of making arrays, to arrays, to using arrays, to devices for making arrays, and to devices for using arrays, among other things. In a particular aspect the invention relates to methods for making arrays by contacting array members with a substrate, activating part of the substrate to bind one or more of the array members with which it is in contact, binding the array member or members thereto, and preparing the substrate to repeat the cycle of contacting, activating and binding and clearing with additional array members, whereby binding array members to different parts of the substrate in succeeding cycles fabricates an array of the array members bound to the substrate. The invention also relates to the arrays thereby formed, to devices for carrying out such methods to form arrays, to uses and ways of using the arrays, to information that can be obtained thereby and

to uses of the information.

An understanding of aspects of the invention may be gain from Figure 1, which shows a process for making arrays in accordance with certain preferred embodiments of the invention. The figure illustrates a process beginning at the upper left with step one (S1) of cycle one (C1), which provides several empty blanks of a substrate, depicted as rectangles 1, 2, 3 ... n). In the next illustrated step of the process, S2, the substrate blanks are contacted with array members shown as stippling in the rectangles. In step three of the illustrated process (S3) an activating process or agent, represented by a horizontal open rectangle is applied to a portion (referred to below as a sector) of the substrate blanks. As a result of activation array members bind activated portions of the substrate and they remain bound when the substrate blanks are cleared of unbound material in step four (S4). Arrays members bound to activated sectors of the substrate at the end of one iteration of this process are illustrated as stippled areas in rectangles 1, 2, 3 ... n, at bottom left in the figure. The cycle of contacting (S2), activating and binding (S3) and clearing (S4) then is repeated a number of times (C2 ... Cq at top), activating different sectors in different cycles, to build up an array. Array members bound to substrate at the end of a second cycle are shown at bottom middle (stippled areas in rectangles 1, 2, 3 ... n at S4 under C2). The array formed after a number of cycles, q, is shown at bottom right (stippled areas in rectangles 1, 2, 3 ... n at S4 under Cq).

Of course the depiction in the figure is illustrative not exhaustive or limitative. For instance, processes of the invention may involve more steps, fewer steps or different steps from those depicted in the figure. Also processes of the invention are not limited to the illustrated shapes, geometries, relative dimensions or relative orientations of the substrate, substrate blanks, activation area, sectors or bound array members among others. And array members contacted with different portions of a substrate or in different cycles generally will differ from one another.

The invention, which is discussed in detail below, is not limited in any aspect to the particulars of the embodiments illustratively set out in the figures or examples. Rather, the invention can be carried out with practically any array

members and/or substrate materials, and it relates generally to any such methods, arrays, and devices. General aspects of the invention in these and other regards, as well as many other particulars of specific embodiments, are described below. However, the discussion herein is necessarily illustrative, and a true understanding of the invention will be possible only by consideration of the disclosure as a whole from the point of view of those skilled in the arts to which it pertains.

ARRAYS

Generally, as to the invention disclosed herein, an array is an arrangement of array members. Often it is convenient to define an arrangement by positions of array members relative to one another in an array. A given arrangement may be defined in this way by the relative positions of some but not necessarily all of the array members in an array. In certain preferred embodiments, array members are in fixed positions in an arrangement.

In one general aspect, the invention is useful to produce replicate arrays all having the same arrangement of array members. In certain preferred embodiments of the invention, array members are disposed in the same arrangement relative to one another in all replicates of a given array. In certain highly preferred embodiments of the invention, the array members have the same fixed positions in all replicates of a given array. In other embodiments of the invention, some or all array elements in individual replicates vary in positions relative to one another. Where the arrangement varies between replicates, in preferred embodiments of the invention, the array members can be identified by other information.

ARRAY MEMBERS

Array members may be anything to be arrayed or arrayed. For instance, to give just a few examples, array members may be atoms, molecules, thin films, ceramics, glasses, metals, polymers, compounds, compositions, gels, mixtures, combinations of the foregoing and just about any other composition of matter. Preferred are those that have or are useful to identify or determine in other

substances physical, electrical, magnetic, electromagnetic, chemical, biochemical, biological and other properties of interest. Examples of preferred embodiments in this regard include those that: bind analytes, absorb light, fluorescence, quench fluorescence, phosphorescence, those that are chemiluminescent, electroluminescent, sonoluminescent, piezoelectric, those that are polymers, metals, alloys, ceramics, organic compounds, inorganic compounds, biomolecules and biomaterials of interest, such as those to be screened for desired properties or those to be used as screening agents for properties of other substances, and combinations of any of the foregoing. Particularly preferred embodiments in this regard include polypeptides, including partial or complete proteins and peptides, polynucleotides, such as DNAs and RNAs, including relatively long polynucleotides and oligonucleotides, compounds that bind to polynucleotides sequence-specifically, such as peptide nucleotide acids and DNA sequence-specific polyamides, polysaccharides, ligands, ligand-binding biomolecules, molecules of pharmaceutical interest, chelating agents or those that bind to chelating agent-derivatives, fractions of cells or tissues, parts of cells or tissues, whole cells, whole living cells, derivatives and modified forms of the foregoing, and mixtures of any of the foregoing, to name just a few. In fact, arrays of the invention are not limited to any particular type of array member, and the foregoing examples, as well as other examples set forth elsewhere herein are necessarily merely illustrative.

Particularly preferred in certain embodiments of the invention relating to binding assays, as described in greater detail herein below, are binding reagents, such as, but not limited to, DNAs, RNAs and other polynucleotides, polynucleotide-derivatives, such as PNAs and other polymeric compounds, such as certain polyamides, that bind to polynucleotides in a sequence-specific manner, antibodies and antibody-derived binding reagents, antigens, ligands, receptor polypeptides and derivatives thereof, aptamers, that bind specifically to cognate compounds or to cognate groups of compounds, such as DNA or RNA aptamers and polypeptide aptamers, to name just a few examples in this regard.

In general, array members may be formed in any shape. For instance,

an array member may be round, oval, ellipsoidal, triangular, square, rectangular, trapezoidal, pentagonal, hexagonal, octagonal, other regular or irregular polygon or any other regular or irregular shape. In preferred embodiments, the array members are uniformly shaped. In other preferred embodiments the array members are homogeneous. In particularly preferred embodiments the array members are both homogeneous and uniformly shaped.

An array member may be formed within or on a substrate or device for forming an array. Thus, for instance, to give but one particular example, an oligonucleotide array member may be synthesized, purified and characterized in advance and then bound to one or more segments of a substrate or device to form part of an array, by a process such as that depicted in Figure 1. The same process can be used as well to synthesize array members on an array support or substrate or device for forming one or more arrays. For instance, the process depicted in Figure 1 can be used to (1) bind to preferably preselected segments an array of first bases for solid phase polynucleotide synthesis; (2) carry out successive deprotection and addition reactions and (3) carry out deblocking reactions of typical solid phase methods to synthesize oligonucleotides *in situ* in the array. For instance, solid phase oligonucleotide synthesis can be carried out on a glass or silicon according to a process much like that depicted in Figure 1 using light-mediated, positionally defined, deprotection to build up oligonucleotides of defined sequences at defined positions in an array. To mention just one other example in this regard, peptide array members also may be similarly synthesized. In addition, array members can be attached to segments in a precursor form and altered into final form after attachment. Array members can have a wide variety of sizes and spacing, including but not limited to the following.

In preferred embodiments of the invention, for instance, there can be 10-100, 50-250, 200-800, 500-1,000, 750-2,500, 2,000-4,000, 2,500-7,500, 5,000-10,000, 7,500-15,000, 10,000-50,000, 25,000-75,000, 50,000-150,000, 100,000-300,000, 250,000-750,000, 500,000-1,500,000, 1,000,000-3,000,000, 2,500,000-7,500,000, 5,000,000-15,000,000 array members in an array.

Particularly preferred are 100-1,000, 1,000-5,000, 5,000-10,000, 10,000-

50,000, 50,000-100,000, 100,000-500,000, 500,000-1,000,000, 1,000,000-10,000,000 and more than 10,000,000 array members in an array. Especially particularly preferred are less than 1,000, 1,000-10,000, 10,000-100,000, 100,000-1,000,000 and more than 1,000,000 array members in an array.

In preferred embodiments array members have cross-sectional areas of about 0.0025-0.0075, 0.005-0.015, 0.01-0.03, 0.025-0.075, 0.05-0.15, 0.1-0.3, 0.25-0.75, 0.5-1.5, 1.0-3.0, 2.5-7.5, 5.0-15, 10-30, 25-75, 50-150, 100-300, 250-750, 500-1,500, 1,000-3,000, 2,500-7,500, 5,000-15,000, 10,000-30,000, 25,000-75,000, 50,000-150,000, 100,000-300,000, 250,000-750,000, 500,000-1,500,000, 1,000,000-3,000,000, 2,500,000-7,500,000, 5,000,000-15,000,000 and 10,000,000-30,000,000 μm^2.

Array members can be spaced in arrays to suit a variety of applications. Preferably in many applications array members are spaced about 0.05-0.15, 0.1-0.3, 0.25-0.75, 0.5-1.5, 1.0 -3.0, 2.5-7.5, 5.0-15, 10-30, 25-75, 50-150, 100-300, 250-750, 500-1,500, 1,000-3,000, 2,500-7,500 or 5,000-15,000 micrometers apart.

The density of array members in arrays preferably is about 10-100, 50-250, 100-350, 200-400, 150-750, 500-1,000, 750-2,500, 2,000-4,000, 2,500-7,500, 5,000-10,000, 7,500-15,000, 10,000-50,000, 25,000-75,000, 50,000-150,000, 100,000-300,000, 250,000-750,000, 500,000-1,500,000, 1,000,000-3,000,000, 2,500,000-7,500,000 or 5,000,000-15,000,000 array members per square centimeter of cross sectional surface area of the array.

In some preferred embodiments, such as flow through embodiments, the flat cross sectional surface area of arrays are much different than the total surface area. In preferred embodiments in this regard, preferred density of array members in the arrays is about 10-100, 50-250, 200-800, 500-1,000, 750-2,500, 2,000-4,000, 2,500-7,500, 5,000-10,000, 7,500-15,000, 10,000-50,000, 25,000-75,000, 50,000-150,000, 100,000-300,000, 250,000-750,000, 500,000-1,500,000, 1,000,000-3,000,000, 2,500,000-7,500,000 or 5,000,000-15,000,000 array members per square centimeter of total surface area. Array members also can be of a variety of depths. In preferred embodiments array members are 0.1-0.3, 0.25-0.75, 0.5-1.5, 1.0 -3.0, 2.5-7.5, 5.0-15, 10-30, 25-75, 50-150, 100-

300, 250-750, 500-1,500, 1,000-3,000 or 2,500-7,500 micrometers deep.

SUBSTRATES

Substrates for forming arrays in accordance with the invention can be formed of a wide variety of materials, as discussed in the "Materials" section below.

In general, substrates for forming arrays may have any shape. Cross sections of channels in certain preferred embodiments described in detail elsewhere herein may be, for instance, round, oval, ellipsoidal, triangular, square, rectangular, trapezoidal, pentagonal, hexagonal, octagonal and other regular or irregular polygons or any other regular or irregular shape. Such channels, moreover, can have one lumen, a few lumen, such as 1 to 10 lumen, or many lumen, such as 11 to 100 or 101 to 200 or 201 to 500 or 500 to 1,000 or more than 1,000. The particular shape employed, the number and size of lumen, the disposition of any coating and of array members with respect to a structural member can be adjusted to best suit a given application and array. Substrates and parts thereof for binding array members can also can be of practically any size, which typically is dictated by the desired size of the array members in the array.

SUBSTRATE MATERIALS

Virtually any material can be used for making arrays in accordance with the invention. The choice of materials for a given array will depend on details of the array members, how they will be mounted and used, and other details relating to specific embodiments in specific circumstances.

In general, materials suitable for use in the invention include any materials that can be shaped into a desired form. Generally, materials that are relatively easy to form are preferred. Particularly preferred are materials that can be shaped to very high precision and very small feature sizes. Thus, particularly preferred materials possess the ability to form a desired configuration with specified dimensions and properties, including, but not limited to desired thickness and density (including having channels); the ability to bind array

members; and tolerance for various treatments including for instance, those associated with making arrays and those associated with use of arrays. Among such particularly preferred materials are those that can be formed into long ribbons or films, such as those of embodiments described more specifically elsewhere herein. Also preferred are materials that can be wafered conveniently, with high precision and close tolerances, and in some aspects of the invention very small geometries. Among particularly preferred materials, as well, are materials that are compatible with array members. That is, materials that do not deleteriously interact with or affect array members during the processes or uses of array formation, including but not limited to clearing, loading, activating and binding array members to sectors on substrates or devices, such as channels, particularly channels in ribbons or films as described elsewhere herein, to other conditions to which arrays may be subjected during manufacture processing, assembly, production, storage, shipment or use. Additionally preferred materials are those that are compatible, in much the same regard, with downstream process steps and end uses. In one aspect in this regard, materials with a high degree of dimensional stability are preferred. In another aspect, materials resistant to solvents and materials used in downstream process steps and end uses are preferred. In another aspect in this regard, materials are preferred that facilitate detection, particularly for analyte detecting and/or determining applications of the invention.

Clearly, the invention can be practiced in many ways and it is not possible to elaborate here more than a few illustrative embodiments. Those skilled in the pertinent arts, having understood the invention, by taking into account such general considerations as those set out above and others that may be pertinent, should be able to choose appropriate materials of practically any sort that will be effective to carry out the invention in any particular application and circumstance. A few illustrative particulars are set out below as further guidance in this regard.

Particularly useful materials for making arrays in accordance with the invention include glasses, plastics, ceramics and metals. Among these glasses and plastics are particularly preferred, especially plastics. Moreover, particularly

for glass and some plastics, many surface treatments and chemical derivations are well known that can be used to bind array members to substrates Similarly, many surface treatments and derivations are known for these materials that can be used for other aspects of the invention, such as detection.

Glasses are particularly preferred for the invention in this respect. A variety of well known fabrication techniques can be used to shape a glass into configurations for use in the invention. Likewise, a great many available glass formulations, treatments and chemical modification techniques readily can be employed in the invention. Thus, to mention just a few types of glasses: standard glasses, functionalized glasses and glass-ceramics all may be used in the inventions. Useful information in this regard is provided in *The Biomedical Engineering Handbook*, J.D. Bronzino, ed., CRC Press, 1995, particularly at pages 566-580, which is herein incorporated by reference in part pertinent to using glass in the invention.

Useful plastics and/or polymers include, among a great many others, polycarbonate, polyethylene, methylmethacrylate, polypropylene, polyester, (poly)tetrafluoroethylene, (poly)vinylidenenedifluoride and the like. Many other plastics and polymers that can be used in the invention are well known to those skilled in the art, such as those described in, among other well known references, *Modern Plastics*, Encyclopedia '97, Volume 73, Nov. 1996 and *The Biomedical Engineering Handbook*, J.D. Bronzino, ed., CRC Press, 1995 (in which, pages 581-610 are particularly useful in this regard), both of which are herein incorporated by reference in their entirety in parts pertinent to using plastics and/or polymers in the invention.

PROCESSING

In some aspects of the invention certain preferred embodiments relate to processing substrates and/or wafers and/or arrays and/or array members before and, during or after array formation to alter, among others, one or more dimensions, one or more structures and/or one or more properties of a substrate and/or one or more array members and/or an array. In certain preferred embodiments in this regard substrates, array members and/or arrays are

processed to alter dimensions of wafers comprising arrays and/or arrays and/or array members. In particularly preferred embodiments in this regard substrates are made of one or more materials that can be formed readily, preferably under relatively mild conditions. Especially preferred in this regard are materials that deform uniformly and/or homogeneously so that their dimensions change uniformly and proportionately. For instance, in certain preferred embodiments in this regard a film comprising channels of given, relatively small dimensions, can be formed from an appropriately dimensioned stock of appropriate larger dimensions.

WAFERS

When multiple arrays, such as multiple unit arrays, are formed together on and/or in and/or within a substrate it may be desirable to separate them from one another into several groups or individual arrays. In additional, whether arrays are made one or many at a time, it often is desirable to remove the array or arrays from extraneous portions of the substrate or from other material at some stage in or following a manufacturing process. Generically, the pieces that result are referred to as wafers in the following discussion.

Wafers can be made in just about any given shape or geometry. Faces, sides and facets of the wafer also can be formed with just about any shape, geometry and/or texture. Wafers can be formed from substrates directly into a desired geometry or first formed in one shape and then modified. Sides, faces, facets, surfaces and other features of wafers may be uniform or not. They may be flat or they maybe contoured in any one or more of a wide variety of shapes. For instance, wafers may be made with one or more surfaces that are, for instance, rippled, grooved, trenched, roughened, polished and the like. In certain aspects of the invention in this regard preferred embodiments provide regularly shaped wafers comprising on one face one or more arrays, each array being disposed on a uniform flat surface.

Production and modification of wafers and wafer surfaces, such as faces, sides and facets can be produced and/or modified by cutting, milling, drilling, forming, abrading, smoothing, pulling, extruding and other forming techniques

well known to the fabrication arts, particularly techniques used to shape, form and finish metal, ceramic, glass and plastic. Such techniques are to employed in accordance with the invention generally so as not to adversely affect either the structure or the performance or arrays or of array members.

DICING

Often a number of arrays formed on a substrate are separated from one another for further processing or for use. Many methods can be employed for this purpose in accordance with t the invention in this regard. For example, the units or groups of units may be cut apart from each other, using any one or more of a wide variety of suitable cutting techniques. Also, substrates may be designed to facilitate later separation of unit arrays or of groups of unit arrays. For instance, substrates may be formed with perforations, for instance, between units or groups of units that will be separate. Alternatively or in addition, for another example, grooves or troughs may be incorporated between units or groups in the substrate to facilitate separation.

A wide variety of cuting methods and devices may be used for this purpose, In conjunction with facilitating structures or without their aid, A number of parameters generally should be considered in choosing the method to use in a given application and circumstance. First, the cutting method should be effective for all the materials in the array. The cutting method should be compatible with the array and the substrate materials, particularly the array members and any other components important to further processing or to using the array being produced. Methods that minimize waste are particularly preferred. Thus, where mechanical cutting is employed, methods that minimize kerf are preferred in this regard. Methods that minimize contamination are preferred as well. Cutting methods that do not foul or close openings are preferred. Generally, preferred cutting methods also minimize environmental stresses, such as heat, that often results from cutting and forming processes. Likewise preferred are methods that avoid altogether or minimize exposing the units or substrate abrasives, lubricants, solvents, coolants or the like, especially those could deleteriously affect the array members, the arrays or functional

aspects thereof.

The following discussion of more specific cutting methods further illustrates this aspect of array production in accordance with the invention. In general, methods that can cut with greater precision are more highly preferred than less precise methods. In particular, for many applications and embodiments, the thinner the sections that can be produced the better. In this regard, low vibration, positionally stable cutting methods are preferred.

Mechanical methods for cutting, among other things, glass, plastics, ceramics and metals are well known. Well know cutting devices that can be used for dicing to produce wafers comprising groups of unit arrays, unit arrays and the like include knife-edge devices. Among these microtome knives and cutting devices of similar sharpness are particularly useful. Generally, microtome cuts do not produce discernable contamination across the cut surface and cut without distorting shape. Microtome knife edges make uniform cuts both as to individual cuts and serially from cut to cut. Generally the cuts do not generate heat and they do not require lubricant, solvents or cleaning solutions.

Ultra high pressure liquid cutting is another useful technique for cutting to produce or process wafers in accordance with the invention. Ultra-fast jet streams of liquid, such as those used to cut steel and other metals, provide a clean, precise, temperature controlled cut that can be quite useful in some embodiments of the present invention. Methods of this type are particularly preferred for cutting glass, metal and ceramics, particularly when microtomes cannot be used.

Laser cutting also is a preferred cutting technique in certain embodiments of the invention. Other mechanical cutting devices that may be useful in the invention include rotary and reciprocating cutting devices, including circular saws, band saws and wire saws, to name just a few. Tools of this type can be used in the invention, in keeping with the foregoing considerations.

The foregoing list is merely illustrative. Clearly, a great many other techniques, can be used to section wafers in accordance with the foregoing requirements including many well known to persons skilled in the pertinent arts.

ILLUSTRATIVE EMBODIMENT OF A PROCESS FOR PRODUCING ARRAYS USING A RIBBON SUBSTRATE COMPRISING CHANNELS ORGANIZED IN BANKS

Many aspects of the present invention are illustrated by preferred embodiments relating to the immobilization of a large number of different array members at discrete locations on a substrate surface, especially by certain particularly preferred embodiments t in which the substrate comprises channels and array members are bound to a surface of the channels. The discussion below relates to especially preferred embodiments in this regard, in which the substrate comprises a plurality of non-communicating channels. It relates to further particularly preferred embodiments in which array members are introduced into the channels, sectors of the channels are activated to bind array members therein disposed, the unbound material is removed from the channels and the cycle of introducing array members, activating and binding, and then clearing the channels is repeated to build up an array. The process is of the type depicted in Figure 1. In addition, the discussion relates to especially preferred embodiments in which arrays are formed on a surface, in particular on one surface of the channels.

The discussion also relates to certain preferred embodiments of the invention for manufacturing arrays of the type depicted in Figure 2, in which the substrate is a long, thin ribbon and many arrays are formed at the same time by reiterating the array formation process both across the substrate and along its length. In the particular embodiments of this type discussed below substrate comprises identical groups of channels across its breadth, and array members are introduced into each group of channels in the same way during each cycle. Similarly, the discussion relates to preferred embodiments in which array forming areas are defined all along the length of the substrate, and sector activation is carried out in each cycle in the same way in all of the array forming areas during each cycle. In this regard, the following discussion relates to preferred embodiments in which the same array is formed in the array forming areas across the breath and along the length of the substrate.

The following discussion is provided for illustrative purposes. It relates to but a few of many possible embodiments of the invention. The dimensions and geometries thus are merely exemplary. Many other dimensions can be used for flow channels, ribbons, activation Lines, spacing, embedded information, manifolds, matrixes, carriers and the like. The choice of dimensions generally will be a function of engineering and design considerations, such as the materials to be employed, the accuracy and precision of tools to be used for manufacturing, the number and type of assays to be mounted and the number of elements they are to contain, the nature of the immobilization chemistry, the methods to be used for activation, the accuracy and precision of the activation method, the assay development techniques to be used, the amount of sample and of reagents required, the nature of the detection scheme, such as the nature of the detectable label, the spacial and quantitative precision and accuracy of the detecting system, and the reproducibility of the overall process in a given embodiment, to name just a few parameters that will effect the design of a given implementation of the invention.

The manifold, the ribbon and the exposure areas are used to form chips that contain 400 different array members (referred to in the following discussion as AMs) in the following manner. (1) 40 AMs are introduced into the 40 ports in the manifold and distributed to the same position channel In each group of 40 flow channels in the ribbon. The AMs are in solution or in a gel and are uniformly distributed along the length of each flow channel in the ribbon. (2) After a first group of 40 AMs is introduced into the flow channels the first exposure region is exposed to an activating agent, typically light. Exposure to light activates one of the surfaces of the flow channel to form covalent linkages to the AMs in the flow channels. (3) Unbound AMs are recovered from the channels. (4) The channels then are thoroughly purged of non-covalently bound AM. A second set of 40 AMs is then introduced into the channels and immobilized by photo activated cross-linking area in the channels. The process is repeated until AMs are immobilized in all the exposure areas, 10 times for the 10 exposure areas in this example. This process builds a chip having many different AMs covalently linked to its surface by a process that involves repeating

10 times the relatively simple steps of introducing 40 AMs into the manifold, activating the surface in an exposure area, and washing the channels. A complete set of exposure cycles in this example produces chips .2 by 8 millimeters, since there are 10 exposure areas of 200 micrometers (with spacing) each along the length of the chip and 40 flow channels of 200 micrometers each across the width of the chip (150 micrometers with a 50 micrometer barrier). Each chip can be made with 400 different AMs in 400 different positions.

As shown in Figure 2, for manufacturing the process is carried out in such a way that many assays are formed at the same time. AMs are introduced into the flow channels via a manifold. The illustrative manifold of this example has 40 ports, one for each of the 40 channels that form the group of channels that go into a chip. Each port connects through the manifold to all of the channels in corresponding positions in each group of 40 channels. That is, the first port connects to all the first channels in every group of forty channels. That way, the first port distributes the connected reagent into all the corresponding channel in all the groups across the ribbon. The same is true for the other ports in the manifold. Thus, each set of 40 reagents gets distributed through the manifold in the same order into each group of 40 flow channels across the width of the ribbon. Accordingly, the order of AMs in the channels will be the same in every chip formed from the ribbon. A simple manifold in this regard can be made by forming the ribbon with flow channels from a much larger dimensioned starting blank, leaving both ends of the blank larger than the ribbon so that it is relatively easy to "wire" the appropriate connections. An augmented approach of this type provides a "macro"- dimensioned manifold that mates to a "macro"-dimensioned end of the starting blank. Alternatively, the manifold may be designed to connect directly to the flow channels in the ribbon, on one "side," and to containers for reagents, such as AMs, buffers and the like, on the other "side."

The ribbon also is figuratively divided along its length into regions defined by an activation line. Activation is accomplished by photochemical means in this illustrative example and the activation line is defined by an illuminating beam that extends across the width of the ribbon perpendicularly (orthogonally) to its length. The beam width defines the size of the activation/exposure region along

the length of the channels. In this example the activation beam defines 10 activation regions of about 150 micrometers each, separated by about 50 micrometers. Thus, the 10 activation Lines and spacing between them defines a region of about 2.0 millimeters along the length of the flow channels. A bank of 40 flow channels together with the 10 activation Lines defines a chip 2.0 millimeters by 8.0 millimeters containing 400 assay areas about 200 micrometers by about 200 micrometers (including spacing) arranged in a 10 by 40 grid. The ribbon can be wider than set out above and generally it will be longer than a single chip length, to facilitate production of a large number of chips. Accordingly, activation Lines can be banked so that each activation step is effectuated in several areas along the length of the ribbon. With banks of flow channels and banks of activation Lines, each cycle of ten activations will produce a grid of chips in a section of the ribbon. Illustrative embodiments of this aspect of the invention are depicted in Figure 2A and Figure 2B. In certain preferred embodiments in this regard, each set of AMs, 40 in this case, is immobilized in the appropriate positions all along the length of the ribbon before it is removed. Thus, each AM needs to be introduced to the ribbon only once during the chip-making process.

In the illustrative example described here, a surface of the flow channels is photosensitive and when exposed to light of appropriate wavelength and intensity it forms covalent bonds with the AMs in the flow channels binding them. The exposure to the activating light in this example therefore photo lithographically defines the pattern of AMs immobilized in the flow channels.

An example reaction scheme for forming and using a photo-activatable surface in this regard employs commercially available alkylamine-polystyrene plastic and commercially available cross-linking agents. A wide variety of photo-cross-linking agents that can be used in this regard are well known. To name just one among a great many texts and other publications in this regard, many such immobilization chemistries and procedures that can be used in this invention can be carried out as described in, or analogously to, e.g., U.S. Pat. No. 5,965,106, 5,268,306, Hermanson *et al.*, IMMOBILIZED AFFINITY LIGAND TECHNIQUES, Academic Press, San Diego (1992), which are incorporated by

reference herein in its entirety, particularly in parts pertinent to immobilization chemistries for use in activation processes as described herein.

Various crosslinking agents can be utilized, including, e.g., photoactivatable arylazide-containing hetero-bifunctional reagents with an amino-reactive NHS ester include N-hydroxysuccinimidyl-4-azidosalicylic acid, (NHS-ASA), N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (sulfo-NHS-ASA), sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (sulfo-NHS-LC-ASA), N-hydroxysuccinimidyl N-(4-azidosalicyl)-6-aminocaproic acid (NHS-ASC), N-hydroxy-succinimidyl-4-azidobenzoate (HSAB), N-hydroxysulfo-succinimidyl-4-azidobenzoate (sulfo-HSAB), sulfosuccinimidyl-4-(p-azidophenyl)butyrate (sulfo-SAPB), N-5-azido-2-nitrobenzoyloxy-succinimide (ANB-NOS), N-succinimidyl-6-(4'-azido-2'-nitrophenyl-amino)hexanoate (SANPAH), sulfosuccinimidyl-6-(4'-azido-2'-nitrophenylamino)-hexanoate (sulfo-SANPAH), N-succinimidyl 2-›(4-azidophenyl)dithio!acetic acid (NHS-APDA), N-succinimidyl-(4-azidophenyl)1,3'-dithiopropionate (SADP), sulfosuccinimidyl-(4-azidophenyl)-1,3'-dithiopropionate (sulfo-SADP), sulfosuccinimidyl-2-(m-azido-o-nitrobenzamido)ethyl-1,3'-dithiopropionate (SAND), sulfosuccinimidyl-2-(p-azidosalicylamido)-ethyl-1,3'-dithiopropionate (SASD), N-hydroxysuccinimidyl 4-azidobenzoylglycyltyrosine (NHS-ABGT), sulfosuccinimidyl-2-(7-azido-4-4-methylcoumarin-3-acetamide)ethyl-1,3'-dithiopropionate (SAED), and sulfosuccinimidyl-7-azido-4-methylcoumarin-3-acetate (sulfo-SAMCA).

The chip may be used alone or with other, independently manufactured chips. The grid depicted in the illustrative drawing contains 6 sub-assemblies of 5 chips each, for a total of 30 chips and 12,000 assays. In fact, modularity provides important advantages. For instance, since each chip in an assembly is made independently of the others one manufacturing run can be used to make up for failures in a previous run.

Also, activation need not be uniform on the exposed surface of the flow channel. Exposure can be varied in intensity in different areas. These different areas are illustrated as "elements" of Figure 6. Each exposure area thus may be made of many elements coated with different concentrations of an AM, also figuratively illustrated in Figure 6.

Chips also generally can contain borders between elements and/or assay areas and areas associated with elements, assay areas or generally with the chip as a whole, such as area for embedding markers and information. Some embodiments in this regard are illustrated in Figure 6. As described elsewhere herein, the data may be imprinted during manufacture of the ribbon or may added to the ribbon after manufacture. Moreover, information may be encoded by or with the immobilization of the AMs in the flow channels themselves. Information may be in the form of pseudo AMs that are immobilized along with AMs or independently. Such pseudo AMs can provide information for registration, alignment, detection, internal control, calibration and the like.

The ribbon can be quite long and, therefore, well-suited to mass manufacturing processes. Some features of an approach to producing large numbers of chips by this process using a long ribbon substrate and photo-activation are illustrated in Figures 1 and 2, Figures 3A, 3B and 3C and Figures 4A and 4B. Further details relating to the method are depicted in Figures 6, 7 and 8.

Figures 1 provides a general schematic of the array forming process of this example as discussed in more detail elsewhere herein. Figure 2 depicts a method implementation for forming many arrays at the same time in a long ribbon substrate, also as discussed in greater detail elsewhere herein. Figure 3A shows a device for photo-activating a ribbon substrate of the type discussed in this example. Figures 3B and 3C show two ways to reposition the activating lines along the length of the substrate. These are discussed in greater detail below.

Figure 4A depicts a ribbon spooled at both ends of a device for activation and for quality control. The ribbon can be run over the activation platform in either direction with equal facility. The illustrative embodiment in the figure includes a means for moving the ribbon so that it can be exposed in numerous positions along its length from one end to the other. The ribbon can be unspooled and respooled on the drums at each end of the device so that each reagent for immobilization need be introduced once, and so that several groups of reagents can be introduced successively into the ribbon, as described

elsewhere herein. For photo activation in this illustrative embodiment, the device contains several illumination stations that comprise a light source, mask holder, clamping means to hold the ribbon in place, optical assembly, exposure indicator and positioning means, among other things. An illuminator of this type is shown in greater detail in Figure 3A. The platform also contains quality control modules for determining efficiency and uniformity of covalent bonding of each AM to each exposure area. In addition, the spools contain ribbon manifolds for adding solutions.

The system is parallelized for high throughput. For instance, the illustrated station could be on that illuminates 20 corresponding exposure areas at the same time. It operates in the following manner, much as described above. The ribbon and manifolds are mounted on the spools. 40 AMs are introduced into the 40 ports in the manifold, filing all 20 banks of 40 flow channels in the ribbon. The entire length of the ribbon is wound through the platform and passed over the QC units to identify inhomogeniety or irregularities in the distribution of the AM anywhere in the ribbon. When the distribution is even in all the channels, the ribbon and illumination means are moved so that the 20 first areas in the first 20 sets of 10 exposure areas each are properly positioned for surface activation. Following activation, the ribbon and illumination means are moved so that the 20 first areas in the next 20 sets of 10 exposure areas each are properly positioned for surface activation. This process is repeated until the first set of 40 AMs is properly immobilized in all the appropriate positions along the length of the ribbon. Then the same process is repeated with nine successive sets of 40 reagents in each set.

It may be useful to note that the number of times activation must be carried out for a given fill of 40 reagents depends both on the ribbon length and on the number of Lines in the bank of activating Lines. Giving each chip 2.0 millimeters along the length of the ribbon there would be 10,000 chip regions along a ribbon 20 meters long. If there are 20 activation Lines working on 20 of these regions at a time, there will be 500 activation positions for each fill of the ribbon with forty reagents. Notably, while the number of repositions for a given configuration goes up with the length of the ribbon, the associated steps of filling

and washing and the like do not change. AM binding can be assessed by QC units for every exposure. Abnormalities can be corrected or recorded and saved for use in later stages of the manufacturing process. To facilitate QC in some embodiments of the invention, the liquid in the flow channels can be moved back and forth. Such motion can be used to check for bubbles or inhomogeniety of the fill. It can be used to distinguish surface bound AM from AM in solution. Tags can be included in the solutions for this purpose, such as tags that bind to activated surfaces. Non-immobilizing taggant can be used to facilitate monitoring liquid flow in the channels. Labeled AM can be spiked into the AM for immobilization, likewise to monitor and control liquid flow in the channels, the immobilization reaction and the immobilization of the AM.

When the first 40 AMs are bound to all the intended positions in the flow channels they are removed from the ribbon. These reagents could be recovered -- in fact in some applications, for instance, where the process does not affect the AM solution and the amount of AM removed by immobilization is relatively small and does alter the concentration, the AM solution can be recovered and reused without further processing. After the first set of AM reagents is removed, the flow channels are thoroughly washed. Then, a second set of 40 AMs is introduced into the flow channels and they are bound to activated surfaces in the same way as the first set of 40 AMs. The second set of AM reagents then is removed and the channels are washed again. The process is repeated ten times, until all 10 exposure areas in each set of ten exposure areas have been activated and coated with an AM.

After the final wash a coating solution or gel may be introduced into the flow-channels to protect the AM surface from dessication or mechanical damage during the following stages of the manufacturing process.

After the last wash step or at the end of the coating procedure, the ribbon is removed from the spools and the manifold. Next, the guide is removed from the AM surface. Protective surface coating may occur at this stage rather than or in addition to the early coating step. Protective coating may be done as the guide is removed from the AM surface or thereafter.

The ribbon is then diced to provide assay surfaces for detection devices.

The ribbon may be diced to provide unit assay surfaces, or it may be diced to produce larger assay surfaces that contain redundancies. In the illustrated example the ribbon is cut in unit assay surfaces that are 2 millimeters by 8 millimeters. Dices of this size are relatively easy to handle, manually or by automated devices. The dice pieces generally will be mounted in a carrier, which may be designed to accommodate one or more dice pieces. The dice pieces in a carrier may be the same or different types. Generally, several different assay surfaces will be mounted together in a carrier. Mounting several dice pieces in each carrier can serve to increase the number of assays in the final device, provides for redundancy and provides several opportunities to mount a successful manufacture of each intended assay, so that the failure of any one assay in a manufacturing run does not necessitate discarding the entire product from that run. This also facilitates redundancy so that each carrier can have all the assays it was intended to have even if the manufacture of an assay in a particular ribbon was faulty. The embodiment illustrated in Figure 6 has 30 chips mounted together in a matrix of 6 5-chip sub-assemblies. This configuration provides 30 chip-level opportunities to succeed in manufacturing any given assay. Assuming a failure rate of 20% overall, the 30 chips in the illustrated embodiment can comprise over 9,500 different assays that meet quality control requirements.

Ribbons suitable for use in the processes described above and elsewhere herein can be made in a variety of ways. A preferred embodiment in this regard, but only one of many effective ways to form ribbons for use in the invention, is illustrated in Figure 5. As depicted in the figure, the flow channels are formed by bonding two thin films together. One film has open-ended channels and serves as a channel former. The other thin film in this illustrative example is a flat surface. The production of thin films of these types is a highly developed industrial process practiced on a vast scale by many industries. Such films are produced for photography for instance. And, the same coating techniques used to make photographic film can be used to coat one or other or both surfaces for use in accordance with the present invention. The production of ultra small ridges in a film to make a channel forming film also is known. Several

companies market such films. Standard techniques for manufacturing thin films may not be suitable to the production of ribbons in which the flow channels have very small dimensions. Such films can be produced using molds and forming devices made by micromachining techniques, such as MEMS, and other such techniques. Presently available techniques in this regard that routinely are able to machine feature sizes below 1 micrometer can be used for this purpose in accordance with the present invention. In fact, techniques used to mass produce the ubiquitous audio compact disc by, *inter alia*, injection molding polycarbonate, routinely achieve precise feature sizes of about 700 nanometers, and are readily applicable to making ribbons for use in this invention.

The amount of an AM needed for this process to make a large number of chips is quite small. For instance, a flow channel lumen 150 micrometers wide, 200 micrometers high and 20 meters long has a volume of about 0.6 milliliters. Filling such a flow channel with a 5 µg/ml solution of an AM would require 3 micrograms of an AM. 20 flow channels in 20 banks across a 20 meter ribbon would require 60 micrograms of the AM. 60 micrograms thus would be enough to produce 200,000 chips that take up 2.0 millimeters along the ribbon length (such as the 2 by 8 millimeter chip with 400 assays described above).

Generally, this estimate greatly exaggerates the amount of material, such as an AM, required to produce chips. First, for antibodies, for instance, most immobilization protocols call for concentrations of less than 5 µg/ml. Indeed, it is estimated that 1 µg/ml for most proteins is the highest concentration that will form a monolayer on a surface, since higher concentrations lead to protein-protein binding and other effects that interfere with monolayer formation. For example in this regard see Cantarero *et al.*, *Anal. Biochem.* 105: 375 (1980), which is herein incorporated by reference in its entirety. Thus, the foregoing estimate is 5-fold too high on this basis alone.

Furthermore, the foregoing estimate does not take into account the possibility of recovering the AM-containing solution, rather than using it only once and then discarding it. In fact, the amount of AM that bonds to the flow channel surface will be a very small fraction of the AM in the solution. For one thing, although a flow channel contain the solution throughout, only one of ten

exposure areas in the channel is involved in binding the AM. In the foregoing illustrative embodiment with 10 activation Lines per chip area, only 10% of the surface will be activated for each fill; 90% of the AM-containing solution will lie above the inert areas of the surface. Accordingly, 90% of the AM in the flow channel will remain unaffected by the immobilization process, for this reason alone. If the solution is recovered from the flow channels after the immobilization process, and the AM in the solution can be recycled to another fill, the foregoing estimate is about reusing the solution 11 times, so that most or all of the antibody in the solution ultimately is immobilized would greatly reduce the amount of an AM required to manufacture large numbers of chips.

However, even this estimate is too high because it does not take into account that only a small fraction of the AM in the solution above the exposure area will form covalent bonds with the activated surface. (One way to estimate the desirable amount of antibody that to immobilize is by reference to conventional solid-phase antibody-based assays.) A typical ELISA immobilization procedure binds about 10 nanograms of an antibody to the surface of a standard microtiter plate well from 100 µl of a 1 µg/ml antibody solution. The surface area contacted by 100 µl in the well is about 100 mm^2. (See, for instance, Table 2 on page 39 in Kemeny *et al.* (1988) *supra*.) This amounts to 100 picograms per mm^2.

For a ribbon that yields 10,000 chips with 400 assays and the dimensions discussed above, each assay area will occupy a total of 400 mm^2. Given a loading of 100 picograms per mm^2, the 10,000 chips would require 40 nanograms of each AM. This a small fraction of the 60 micrograms introduced into the channels, as discussed above.

It may be noted that 200 nanograms is 1.3 picomoles of an antibody of 150,000 daltons. This amounts to 8×10^{11} molecules. If the binding yield of detectable label in an average assay is 0.01 % of the surface antibody, without amplification an average assay will provide about 8×10^8 detectable labels. The density will be 1.6×10^{10} per square millimeter.

ILLUSTRATIVE EXAMPLE ARRAYS FOR ANALYE ANALYSIS

The following discussion illustrates various aspects of the invention, by reference to certain specific embodiments useful to detect and, in some cases quantify, analytes in samples. The discussion is merely illustrative and discloses general features of the invention solely by way of specific examples. The invention is not limited in any way to particulars or details of these examples. For instance, the invention is not limited to analyte detection or quantification using analyte binding reagents, as is the discussion in these examples. Likewise, the invention is not limited to the particular assays or array formats discussed below. Rather the examples merely exemplify a few preferred embodiments of the invention and thereby illustrate aspects of its more general features.

Detecting and quantitating substances of various sorts in a wide variety of samples are important components of many economically important activities. For instance determining analytes, such as molecular, macromolecular and cellular analytes is important in biological, chemical, veterinary, clinical, forensic, agricultural, food, environmental, consumer products, process stream, quality control, military-related and other types of samples. Arrays, array-based devices, and array-related methods and apparatuses can be used for analysis of analytes in all of these types of samples, among others. Analyte determining arrays can be used in this regard for, to name just a few examples, clinical and veterinary diagnostic analyte analysis, forensic analysis, food quality monitoring, agricultural monitoring, environmental monitoring, monitoring of microbial agents, chemical and biological warfare agent monitoring, and process control monitoring. The following discussion illustrates the use of the invention in regard to specific embodiments of analyte-determining arrays.

NOMENCLATURE IN THE FOLLOWING DISCUSSION

A variety of terms are used in the following discussion in ways specifically relating to the exemplified analyte-determining embodiments of the invention. The following discussion provides illustrative explanations of some of the terms,

as an aid to understanding the discussion and, thereby, the invention. However, the brief discussion immediately below does not provide exhaustive definitions, and it is not intended to circumscribe limitations of the invention, which can only be understood from careful consideration of the disclosure as a whole in light of related and ancillary knowledge in the arts to which it pertains.

sample Samples suitable for analysis in the present invention include any sample that can be brought into contact with assays in a chip for effective detection or quantification of an analyte. Preferred samples are homogeneous liquid samples. Included are biological fluids such as blood, serum, urine, saliva, spinal fluid, tears, lymph, bile, peritoneal, and wound fluids. Also included are homogenized biological samples such as homogenized mucous and homogenized bowel samples, as well as homogenized biopsy samples. Preferred samples include the aforementioned biological fluids, of which blood, serum, urine and salvia are particularly preferred.

analyte In the following discussion, any molecule, compound substance, organism or other thing that is to be detected or quantified is referred to as an analyte.

ABR In the following discussion, a reagent that binds to and is useful for detecting, quantifying or analyzing one or more analytes is referred to as an analyte binding reagent ("ABR"). Generally, an ABR is any entity that binds to and can be used to detect the presence or determine the amount of an analyte. An ABR and the analyte(s) it binds are referred to as cognates. In some preferred embodiments the ABRs bind their cognate analytes very tightly and with great specificity. In other preferred embodiments, ABRs bind their cognate analytes much less tightly and with much less specificity. Such lower affinity and specificity ABRs are particularly useful in arrays that implement nose-like sensing paradigms. Analytes can be detected and quantified by binding to one or to more than one ABR.

molecule, compound, etc. It will be appreciated that any atom, molecule, complex of molecules, mixture of molecules or molecular complexes, molecular aggregates, macromolecular or other complexes, aggregates, combinations of the foregoing or any other assemblage of matter can be used

in accordance with the present invention if it is useful to the determination of any other assemblage of matter (as illustrated by the foregoing illustrative listing) and it can be incorporated into the devices of the invention or used in the methods and apparatus of the invention herein described. The terms molecule and compound and related terms generally are used herein to refer to any of these.

chip As used below, chip means the same as wafer, and as unit, unit surface and dice.

element Usually the smallest feature on a chip is referred to as an element. Most often it constitutes a defined area that contains a particular analyte binding reagent. The signal from an element may be obtained by sampling and calculation. Thus, for instance, a laser (or other sensing element) for reading an array can be smaller than the array elements, in which case the smallest signal-generating area is defined by the laser, not the array.

assay The part or parts of a chip relating to a given assay or test is referred to as an assay or assay areas. An assay may comprise one or more elements, which may be grouped or distributed in an array. For instance, an assay may comprise a graded concentration of an ABR to provide a quantitative curve of analyte binding. An assay also may comprise negative and positive controls.

autoanalyser An instrument for automatically processing sample test matrixes, generally comprising (1) a mechanism to transport carriers through the instrument, (2) a microprocessor controlled liquid delivery system that delivers appropriate reagents to the test matrix according to its type, (3) a system for digitizing all the assay results within each element in the matrix, and (4) a computerized analysis system to determine the results of each assay, to report the results, and to bill for the requested tests.

GENERAL CONSIDERATIONS FOR ANALYTE ANALYSES USING ARRAYS

The nature of the ABRs, the analytes and the sample(s) in which the analytes are to be determined largely will determine the analytic chemistries that

are used in a given type of assay of the invention. Those of skill will appreciate from the present disclosure how to adapt particular assay systems for use in the present invention. Many analytic chemistries have been described and are well known to those of skill in the art that can be usefully employed in accordance with the present invention. These are described broadly in, for instance, BIOSENSORS, AN INTRODUCTION, Wiley and Teubner, Chichester and Stuttgart (1996), HANDBOOK OF BIOSENSORS AND ELECTRONIC NOSES, MEDICINE, FOOD, AND THE ENVIRONMENT, Erika Kress-Rogers, ed., CRC Press, Boca Raton (1997) and IMMUNOCHEMICAL ASSAYS AND BIOSENSOR TECHNOLOGY FOR THE 1990S, Nakamura *et al.*, eds., American Society for Microbiology, Washington, D.C. (1992), each of which is herein incorporated by reference in its entirety in parts pertinent to analyte analysis procedures and chemistries. Numerous other publications, such as those cited in the forgoing references, set out assay procedures in much greater detail, and are well known to those of skill in art.

The invention provides several features that will be found advantageous in most, or all, types of assays. First, the invention provides a method to analyze many different analytes in a sample all-at-once. As set forth above, the method utilizes very small amounts of ABR to analyze a sample. In the most highly preferred embodiments, very small amounts of sample are used. And where reagents are employed, the most highly preferred embodiments of the invention use very small amounts of these reagents as well.

Similar considerations apply to the sample.

It will be convenient in many cases to employ an autoanalyser to carry out assays. An alternative to liquid or gas reagents delivered by an autoanalyser may be preferred in low use and/or remote environments. In these situations development reagents may be provided as part of the platform which will incorporate a device for controlling delivery of the reagents to the assays effective for properly developing results. In a preferred embodiment of this type the reagents are in a gel in a geometry that provides reagent assay contact effective for proper development when the gel is extruded unidirectionally across the assays following appropriate exposure to sample. It will be appreciated by

analogy that the operation of this embodiment of the invention is akin to the self-developing films that have been marketed by Kodak and Polaroid, among others, although the analogy should not be taken too literally.

STANDARDIZATION

It will be appreciated that there may be differences between the assays in an array. Therefore, each assay in an array may be designed to perform well under certain specified conditions to be employed. For instance, the assays in a given array might all desirably be designed to work well with a given autoanalyzer of limited flexibility and narrow dynamic range of detection. There are many ways to achieve desirable operative similarity of array assays in accordance with the invention, a few of which are described below.

The amount of ABR can be adjusted for each assay to work well within the available dynamic range. In addition, the amount of a detecting reagent or the amount of label in the detecting reagent may be adjusted. For instance, in a sandwich antibody assay, the amount of first antibody immobilized on the surface may be varied for each assay to work well with the expected sample in the available protocols and detection regimes of the analyzer. The amount of second antibody that binds to the antigen also can be controlled, by its concentration in the second antibody mix. Also, the degree of labeling of a detection reagent, such as a fluorescent tag or biotin affinity label on a secondary antibody, can be adjusted to help insure that the signal from each assay is within the working range of the analyze. For example, by reference to sandwich assays using biotinylation, since each first antibody may be separately immobilized, each second antibody can be separately biotinylated, and many assays can be tested simultaneously in a given autoanalyser protocol. It will almost always be possible to adjust the parameters of any particular assay to a standard protocol.

ILLUSTRATIVE ANALYTE ASSAY METHODS

The invention is useful for, among many other things, carrying out any

assay predicated on binding of one member of a binding to the other, such as binding of an analyte binding reagent and cognate analyte. Numerous assays have been described and are well known to those of skill in the art that can be usefully employed in accordance with the present invention. Many such techniques are set out in some detail in, for instance, BIOSENSORS, AN INTRODUCTION, Wiley and Teubner, Chichester and Stuttgart (1996), HANDBOOK OF BIOSENSORS AND ELECTRONIC NOSES, MEDICINE, FOOD, AND THE ENVIRONMENT, Erika Kress-Rogers, ed., CRC Press, Boca Raton (1997) and IMMUNOCHEMICAL ASSAYS AND BIOSENSOR TECHNOLOGY FOR THE 1990s, Nakamura *et al.*, eds., American Society for Microbiology, Washington, D.C. (1992), each of which is herein incorporated by reference in its entirety in parts pertinent to assay methods.

In certain preferred embodiments of the invention, immunological reagents are used, particularly antibody-derived reagents. In other preferred embodiments, oligonucleotide and/or PNA reagents are used. Other preferred embodiments relate to organic affinity reagents (ABRs), such as polyamides that can bind DNAs sequence-specifically. Still other preferred embodiments relate to random "sequence" polymers, such as random sequence DNAs, screened for analyte-binding ability and then used as ABRs.

Some preferred embodiments of the invention relate to high affinity and specificity ABRs, while other preferred embodiments relate to lower affinity and lower specificity ABRs. Particularly preferred regarding low affinity and/or low specificity ABRs are embodiments of the invention in which analytes are determined by patterns of binding to sets of ABRs. Still further preferred embodiments relate in this regard to combinations of ABRs with differing specificity, affinity and avidity for their cognate analytes. Particularly preferred embodiments use information from a variety of such ABRs to determine an analyte.

Illustrative analyte assay methods for use in the invention are described below. However, the illustrative methods are only a very few among many well known assay paradigms that can be practiced in the invention.

Sandwich assays

In a highly preferred embodiment, sandwich assays are used in the invention. In certain highly preferred embodiments in this regard, first antibodies specific for cognate antigens are immobilized in an array, in accordance with methods described herein above. The array is exposed to a sample under conditions effective for antibodies in the array to bind cognate antigens in the sample. Thereafter, the array is washed and then exposed to second antibodies that bind to the cognate antigens under conditions effective for the secondary antibodies to form Ab1-cognateAg-Ab2 sandwiches with cognate antigens bound to first antibodies in the array. The formation of the sandwiches then is detected, thus detecting the presence of the cognate antigens in the sample.

Detection can be qualitative or quantitative. Generally, the second antibodies in sandwich assays are detected independently of the first antibodies. Thus, the first antibodies might be mouse monoclonals and the second antibodies might be goat polyclonals. More conveniently, first and second antibodies both may be mouse monoclonal, but all the second antibodies will have been covalently bonded to a detectable label, such as a fluorescent label. Other labels, of which many are know to those of skill in the art, can be used. Non-diffusing labels are preferable in some situations, where diffusion would preclude accurate localization, for instance. However, solution ELISAs also can be used in some embodiments.

Alternatively, second antibodies may be attached to a chemical hook which subsequently attaches the detectable label. The well known avidin-biotin system can be used in this way. For instance, the secondary antibodies can be tagged with biotin, a fluorescent label can be attached to avidin, and the presence of cognate antigens can be detected by fluorescence of labeled avidin that binds to biotinylated secondary antibodies in Ab1-cognateAg-Ab2 sandwiches formed on the array.

Those skilled in the art will appreciate the wide variety of immunoassay paradigms and the large number of detectable labels that can be used in much the same way as discussed above for sandwich immunoassays and fluorescence or ELISA detection. A general description of many such

techniques that can be used in accordance with the present invention is provided in PRINCIPLES AND PRACTICE OF IMMUNOASSAY, 2nd ed., Price and Newman, eds. Stockton Press, New York (1997), which is incorporated herein by reference in its entirety.

Hybridization assays

Also particularly useful in the invention are hybridization assays and methods for detecting hybridization. Such assays can take a wide variety of formats and are well known to those of skill in the art. Particularly preferred for use in accordance with the present invention are assays that work well in a solid-phase format in which a capture probe is immobilized on a solid phase, such as a surface, and localizes specific hybridization thereto. A number of formats, assay parameters to consider and detectors for chip-based hybridization assays are discussed by Titball and Squirrell in *Probes for Nucleic Acids and Biosensors*, Chapter 4 in HANDBOOK OF BIOSENSORS AND ELECTRONIC NOSES, MEDICINE, FOOD, AND THE ENVIRONMENT, Erika Kress-Rogers, ed., CRC Press, Boca Raton (1997), which is incorporated by reference herein in its entirety. Several formats for hybridization assays are illustratively discussed below; but, many other approaches also can be employed in accordance with the invention.

One approach to hybridization assays is direct detection of duplex formation. Several methods for so-doing are well known to those of skill in the art. Further, several such methods have been implemented in microchips by companies such as Affymetrix and Hyseq. In such approaches polynucleotides in the sample are labeled and their hybridization to the probe polynucleotides (or PNAs) on the chip are detected directly. The labels generally are fluorescent in common implementations of this approach; but, many other types of label can be used, as discussed elsewhere herein.

Another approach to such methods is based on primer elongation, exemplified by the following method for simultaneous analysis of a plurality of polynucleotides in a sample, which comprises the steps of: (A) providing a surface having immobilized in a predetermined pattern thereon a plurality of first

oligonucleotide probes stably hybridizable specifically to one or more polynucleotides, wherein the first oligonucleotide probes provide 3' hydroxyl groups for elongation, and wherein each position in the predetermined pattern exclusively contains a first oligonucleotide probe or mixture of probes specific for a polynucleotide or mixture of polynucleotides of the plurality of polynucleotides; (B) contacting the surface with a sample under conditions effective to hybridize polynucleotides in the sample specifically to the first oligonucleotide probes or mixture of probes immobilized on the surface; (C) removing from the surface components of the sample other than polynucleotides specifically hybridized to the immobilized first oligonucleotide probes on the surface; (D) contacting the surface with a solution comprising a polymerase for primer extension and a detectably labeled polymerase substrate under conditions effective to extend hybrids formed by the probes and polynucleotides of the sample, thereby detectably labeling the hybrids immobilized on the surface; (E) removing from the surface the components of the solution other than the labeled hybrids immobilized on the surface; and (F) determining the detectable label in each position of the pattern on the surface, thereby determining the polynucleotides in sample.

In certain preferred methods of this type the detectable label is a fluorescent label. In other preferred embodiments other types of labels are employed. Also preferred are mass tags for mass spectral detection and analysis. Also preferred in certain embodiments of this aspect of the invention are methods wherein the polymerase is a DNA polymerase and one, two, three or all four of the deoxyribonucleotide triphosphates are detectably labeled with a fluorescent label. In other preferred embodiments in this regard the polymerase is a reverse transcriptase and one, two, three or all four of the deoxyribonucleotide triphosphates are detectably labeled. In certain preferred embodiments in both regards, fluorescent labels are preferred. In another regard, certain preferred embodiments are those in which the T_m or T_d of duplexes formed by hybridization of sample polynucleotides to immobilized probes are within 1, 2, 3, 4, 5 or 10 degrees centigrade of one another. Particularly preferred are those in which the duplexes are within 1, 2 or 3

degrees centigrade under the conditions used for hybridization.

Also useful in the invention are methods for simultaneous analysis of a plurality of polynucleotides in a sample, comprising the steps of: (A) providing a surface having immobilized in a predetermined pattern thereon a plurality of first oligonucleotide probes stably hybridizable specifically to the polynucleotides, wherein the first oligonucleotide probes provide 3' hydroxyl groups, and wherein each position in the predetermined pattern exclusively contains a first oligonucleotide probe or mixture of probes specific for a polynucleotide of the plurality of polynucleotides; (B) contacting the surface with a sample under conditions effective to hybridize polynucleotides in the sample specifically to the first oligonucleotide probes immobilized on the surface; (C) separating from the surface components of the sample other than polynucleotides specifically hybridized to the immobilized first oligonucleotide probes on the surface; (D) contacting the surface with a plurality of detectably labeled and ligateable second oligonucleotide probes stably hybridizable specifically to the polynucleotides, wherein the second and the first oligonucleotide probes are ligateably adjacent when hybridized to a polynucleotide from the samples; (E) separating from the surface, components of the sample other than second oligonucleotide probes hybridized to the polynucleotides hybridized to the immobilized first oligonucleotide probes immobilized on the surface; (F) contacting the surface with a ligase for ligating the ligateably adjacent first and second oligonucleotide probes hybridized to the polynucleotides under conditions effective for ligating the first and second oligonucleotide probes; (G) separating from the surface detectable label other than detectable label in second oligonucleotide probes ligated to the first oligonucleotide probes immobilized on the surface; and (H) determining the detectable label in each position of the pattern on the surface, thereby determining the polynucleotides in sample.

In certain preferred methods of this type, step (G) additionally comprises melting the polynucleotides hybridized to the first and second oligonucleotide probes, whereby detectably labeled second oligonucleotide probes ligated to first oligonucleotide probes remain bound to the surface via the first oligonucleotide

probes immobilized on the surface in the predetermined pattern, and the other polynucleotides are removed.

In still another approach to hybridization assays, the immobilized probe contains two labels that interact through an energy transfer mechanism that is affected by duplex formation. The energy transfer is detectably altered by probe-target binding in a way that enables analyte detection and determination. For instance, the two label can exchange energy by Forster energy transfer. Forster energy transfer efficiency is proportional to the inverse sixth power of the distance between the two labels. Accordingly, it is very sensitive to their proximity. If duplex formation moves the labels even slightly apart energy transfer between them will decline dramatically. The effect is well known to those of skill in the art, and it has been used to measure the relative motion of different parts of various molecules, such as polynucleotides and proteins. Its application in accordance with the present invention can proceed using much the same kinds of techniques, adapted to the solid phase array.

DETECTION

A wide variety of detection techniques may be employed in the invention for reading-out analyte determinations on a chip. The detection techniques, moreover, may rely on any of a wide variety of detectable labels. Such detection techniques and detectable labels are well know and are described in numerous primary publications, technical manuals, laboratory handbooks and textbooks, to name a few. These detection technologies are described in such publications both generally and with respect to specific types of assays and assay procedures. In general, such techniques can be employed in the present invention, particularly those that have already been adapted to detection of binding to genechips and other types of solid-state arrays. Such techniques are described in, for instance, BIOSENSORS, AN INTRODUCTION, Wiley and Teubner, Chichester and Stuttgart (1996), HANDBOOK OF BIOSENSORS AND ELECTRONIC NOSES, MEDICINE, FOOD, AND THE ENVIRONMENT, Erika Kress-Rogers, ed., CRC Press, Boca Raton (1997) and IMMUNOCHEMICAL ASSAYS AND BIOSENSOR TECHNOLOGY FOR THE 1990S, Nakamura *et al.*,

eds., American Society for Microbiology, Washington, D.C. (1992), each of which is herein incorporated by reference in its entirety in parts pertinent to detection.

Generally techniques for detection in accordance with the invention can determine both the presence of a detectable label and its position, relative to other positions in an array on a chip. Both abilities can be attained by a variety of means. For instance, detection may involve acquiring an image of a chip that comprises an image of the detectable label in each element and/or assay area, and then, by image processing, determining the results for each assay. Such image processing may be quantitative, threshold-related or qualitative. Alternatively, the chip may be scanned, raster fashion, or quasi-raster fashion, or along a path (ray-tracing mode) so detection is carried out discretely for each element and/or assay area. Each element and/or assay area thus may be detected one or more times in the same or in different portions, and the detection may involve averaging or otherwise processing such repeated samplings. The sampling furthermore may involve detection of each element and/or assay area along a beam line, such as for an absorption assay, or it may involve stimulation by a beam line and detection of an emitted signal, such as fluorescence. In the latter case, the emitted signal may be measured over a spot or a wide area. In addition, the entire chip (or portions thereof) can be excited *en banc* and the signal coming from elements and/or assay areas then measured individually. Of course, several beams and detectors can be arranged to work in parallel in any such detection paradigm. Spatial resolution may be obtained by tracking the motion and the position of a scanning beam (or other scanning means) as it moves with respect to a chip, by tracking the position of the chip as it moves with respect to the beam, or by a combination of the two. In addition, alignment markers integrated into the chip, sub-assembly, matrix and/or matrix carrier can provide internal alignment and registration signals that can be used by the detector and its associated hardware and software to correlate elements and assays on a chip with the detected signals. Such alignment and registration signals can be incorporated into chips as pseudo-analytes at the time the chip is made. Alternatively or in addition, the markers can be "etched" or written onto the chip outside the elements and the element-

containing portion of the assay areas at the time the chips are made. A wide variety of other approaches also may be usefully employed in the invention. Suitability of detection techniques for use in the invention can be assessed by reference to the following parameters.

The detection accuracy should be high. Preferably, the accuracy is within 2-3% deviation or better about a norm for repeated sampling of the same test result.

For clinical applications, and other real-time applications relatively short read-out times are preferred. Thus, for instance, for clinical and other real time applications read-out times under an hour, preferably less than 15 minutes and especially preferably less than 5 or I minute are preferred.

To accommodate differing analyte concentrations detection techniques that have a wide dynamic range are preferable.

At best, analyte binding to every assay area on a chip can be detected and/or determined for each sample.

The detector must be able to resolve the elements and assay areas, and it must be able to detect the signal in such areas that samples for analysis will provide.

As noted elsewhere herein, they need not be square but can be any shape. Moreover, also as noted elsewhere herein, size is limited not by intrinsic aspects of the methods and systems herein disclosed but by technical considerations and limitations on fabrication equipment, materials and techniques. Those of skill readily will appreciate and apply to the invention improved materials and techniques as they become available. Such application specifically is contemplated by the present invention.

Many detectors for use in the invention presently are available. Suitable detectors range from inexpensive scanners for use with PC's to sophisticated photon counting imaging microscopes. As noted above, different detectors will be preferred in different embodiments of the invention. Thus, for economical mass production greater economy will be desirable. For other applications very high resolution, sensitivity and dynamic range may be more important. The few examples below, therefore, are merely illustrative.

Sophisticated and sensitive apparatus for detection is available that can be used for clinical and other applications in which large numbers of samples are analyzed, or where equipment expense is not as important as sensitivity, speed and accuracy. Thus, for instance, fluorescence detectors of the type used on automated DNA sequences can be adapted for use in the present invention, for detection of one, two, three, four or even more fluorescent dyes. Likewise, the fluorescence detectors described by Affymax and Affymetrics for detecting binding to peptide and oligonucleotide arrays are readily adaptable for use in accordance with the present invention. Likewise, many laser scanning detectors now used for biochip detection, including laser-ablation mass spectroscopy scanning detectors, are readily employed as detectors in accordance with the present invention.

Another approach might be taken for mass producing detectors, that also illustrates some considerations for implementing both simple and more sophisticated detection regimes and systems. This approach, illustratively discussed below, is to use a scanner to capture the results of assays. A variety of scanners are commercially available, largely for digitizing text and drawings for entry in a computer file associated with document production. Nonetheless, the devices are well suited to capturing any image in digitized, computer readable form.

The devices have in common that they scan an image surface with a laser beam and measure the light reflected from the surface along the scan path. The scan line typically is very thin, 1/100 to 1/400 inches in inexpensive scanners. The full image is digitized by serially scanning the image surface in the direction perpendicular to the scan line. Thus, scanners typically move paper in steps after each line scan to build up the two dimensional image of the paper surface. With hand-held scanners the perpendicular movement is performed manually. It will be appreciated that the scanning mechanism is compact and economical in these devices.

A relatively inexpensive hand-held color scanner can resolve 400 dpi in both dimensions, *i.e.,* 16,000 pixels per square inch. Resolution is in three colors with a grey scale range of 256 levels. Consumer devices are available for

digitizing 35 mm color photographs for manipulation on a PC. A typical full color, 12-bit, 2,000 dpi device resolves 4,000,000 12-bit pixels per square inch. More expensive models can achieve very much higher resolution. One device has been reported that can resolve 25,000 dpi, *i.e.*, 625,000,000 pixels per square inch.

EMBEDDED DATA

The detection system can read coded information on the chip in addition to assay test results. Many types of information can be coded, in many formats. One type of information is positional, such as the alignment and registration information discussed elsewhere herein. Such information primarily is useful to allow identification of elements and assay areas with the detected binding, so that signals can be matched to corresponding elements and/or assays. Such information generally can be imprinted at the time the chip is manufactured. Another type of information provides information about the chip itself — its manufacturing lot, for instance. This information, at least in part, can be used to match software to chips so that the results of assays can be calculated and accurately reported. Thus, for instance, the information about each element and assay area in each lot of chips on a given carrier can be recorded in software. The software thus can contain the identity. location, standardization curves and other information about each chip in the carrier necessary for read-out of assay results. The software thus allows great flexibility to chip manufacture, since the location of elements and assays, as well as standardization can be changed from lot to lot in a way that is transparent to the end-user. The ability to identify carriers and chips and match the software to the detected lots provides a way to do this. Such information can include expiration dates for elements, assays and/or carriers that would key an instrument to exclude their use. A third type of "embedded" information can be provided by the user at the time of use, such as a clinician at the time sample is applied. It is highly preferable for the instrument of analysis to be able to capture these types of information, as well as detect the signals arising from analyte binding.

Such information can be embedded in chips, sub-assemblies, matrixes,

matrix carriers, and the like in many ways. It can be incorporated during manufacturing of chips by the use of pseudo-analytes. These are signal-generating molecules, compounds, compositions, materials, substances or the like, that generate a position-specific signal for detection. The signal may be intrinsic thereto or it may arise from the analytical process, along with the signals from genuine analytes. Such embedding may be in elements of an assay area or it may be outside assay areas or both. In fact, it can in principle be anywhere on the chip. Specific areas on a chip may be dedicated to these markers, so that the signals always arise from the same areas on chips and/or the markers can be incorporated into different places on different chips or chip runs. These locations can be dedicated to the markers or they can be areas that could also contain ABRs. Moreover, the signal from the markers can be detected in the same way as analyte binding is detected or it can be detected differently. For instance, one or more colors in a multicolor detection scheme can be used exclusively for marker detection. When such a multiplexing approach is used, the markers may even be incorporated with ABRs in elements of a chip. Particularly accurate alignment can be facilitated by multiplexing.

Such information also can be directly imprinted on chips or within a manufacturing ribbon or other substrate, for instance. Particularly, certain kinds of lot and registration information may be so imprinted. The information may also be incorporated at the time of dicing or wafering.

Information of this type may take many forms, but preferably is machine detectable and readable by computer systems. Thus, the information can be in the form of shapes, bar codes and/or print, that can be acquired and interpreted by the detector and computer analysis methods and systems useful in the invention. Generally well know techniques useful for these purposes can be employed in this regard in the present invention.

What I claim is:

1. A method for making a device having immobilized in a predetermined pattern thereon a plurality of analyte-binding molecules comprising:

a) introducing analyte-binding molecules into a plurality of non-communicating channels comprising one or more surfaces that can be activated to immobilize said molecules;

b) selectively activating a part of said one or more surfaces effective to immobilize analyte-binding molecules thereon;

c) removing unbound analyte-binding molecules from said channels;

successively carrying out steps a), b), and c), each time activating a different part of said one or more surfaces to form an element.

2. A method of claim 1, wherein said channels are formed by joining a first surface to second surface having troughs formed therein, said troughs forming said channels when said first and second surfaces are joined.

3. A method of claim 1, wherein said one or more surfaces is a glass or a plastic.

4. A method of claim 1, wherein said one or more surfaces are photactivatable.

5. A method of claim 1, wherein said one or more surfaces comprise a photoactivatable cross-linking agent.

6. A method of claim 1, wherein said activating is by photoillumination.

7. A method of claim 1, further comprising providing one or more masks for successively carrying said activating.

8. A method of claim 1, wherein said activating is exposing said part of one or more surfaces to a pattern of light defined by a first mask.

9. A method of claim 1, wherein said activating is exposing said part of one or more surfaces to a pattern of light defined by a first mask, whereby said photoactivatable cross-linking reagent is activated in said exposed surface.

10. A method of claim 1, wherein said photoactivatable surface is formed by a process, comprising:

a) coating a surface with alkylamine-polystyrene

b) reacting the amine of said alkylamine-polystyrene with NHS-ASA thereby forming a photoactivatable surface comprising a phenylazide, which is activatable by exposure to UV light.

11. A method of any of the above claims, wherein the analyte-determining molecule is an antibody, an oligonucleotide, a protein-nucleic acid, an aptamer, a ribozyme, a nucleic acid, a peptide, a nucleic acid binding-polyamide, a polysaccharide, a glycoprotein, a lipid, a lectin, a receptor polypeptide, a ligand, an antigen, a fusion protein, a hapten, or a chelating agent.

12. A method of any of the above claims, wherein the analyte-determining molecule is a polyclonal antibody, a monoclonal antibody, a Fab fragment, a single-chain antibody, or a disulfide Fab fragment.

13. A method of any of the above claims, further comprising analyte determination.

14. A method of any of the above claims, further comprising utilizing the device to determine the presence of analytes which comprises: an immunoassay, a hybridization assay, a ligand binding assay, a receptor binding assay, or an affinity binding assay.

15. A method of any of the above claims, wherein the analyte-detection is by radiation, chemoluminescence, phosphophorescence, fluorescence, or energy emission.

16. A device made by a method of claim 1.

17. Use of a device made by a method of claim 1.

18. A method of making a device having immobilized thereon a plurality of analyte binding molecules comprising:

(a) contacting a substrate with array members,

(b) activating a sector of the substrate to form an activated substrate sector, whereby said activated substrate sector can bind array members with which it is in contact; and

(c) binding the array members thereto.

19. A method of claim 18, further comprising (d) removing unbound array members from said substrate.

20. A method of claim 19, further comprising repeating the cycle of contacting, activating and binding and clearing, with additional array members, whereby binding array members to different parts of the substrate in succeeding cycles fabricates an array of the array members bound to the substrate.

21. A method of claim 18, whereby unactivated substrate sector can not bind array members with which it is in contact.

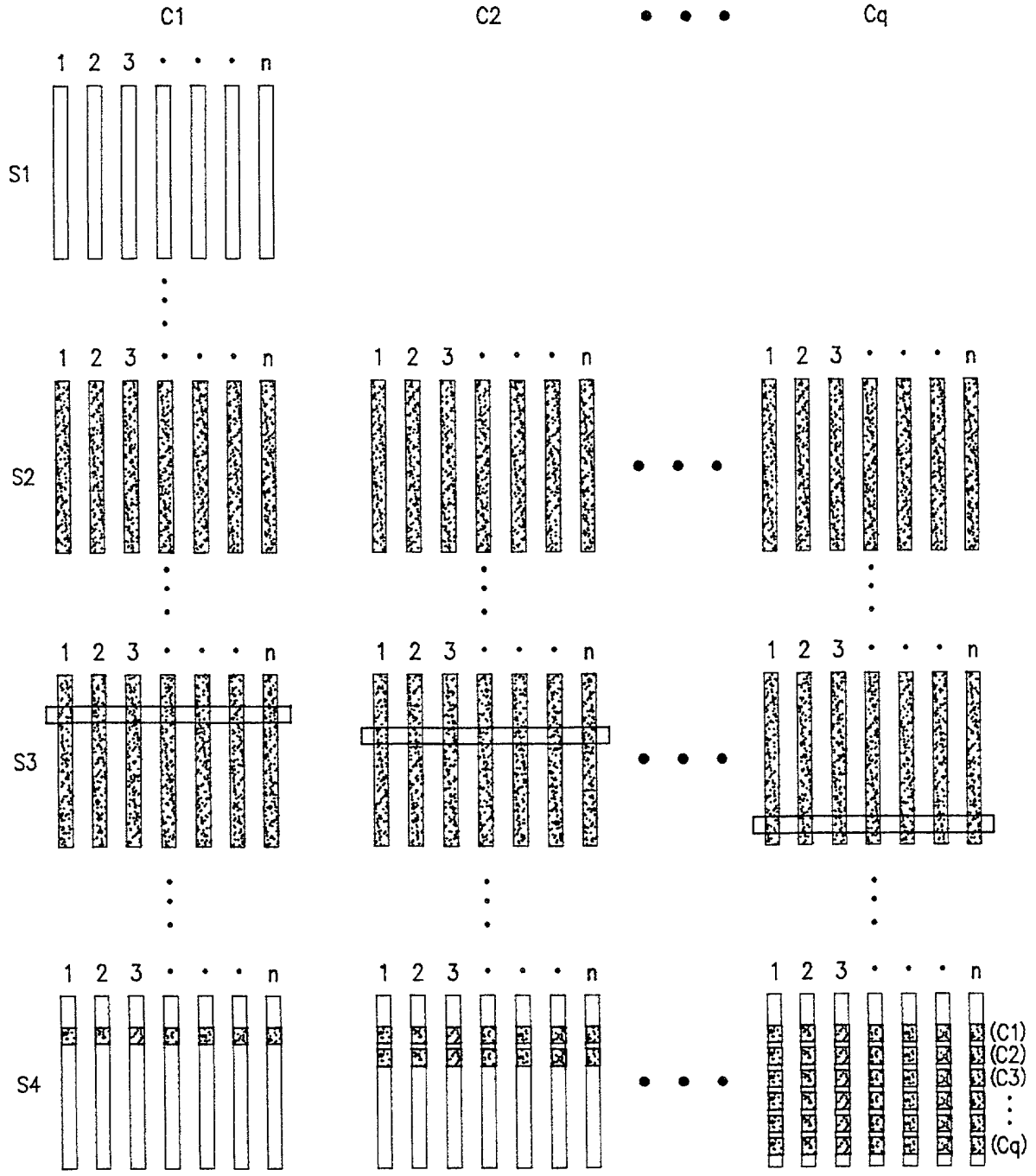


FIG. 1

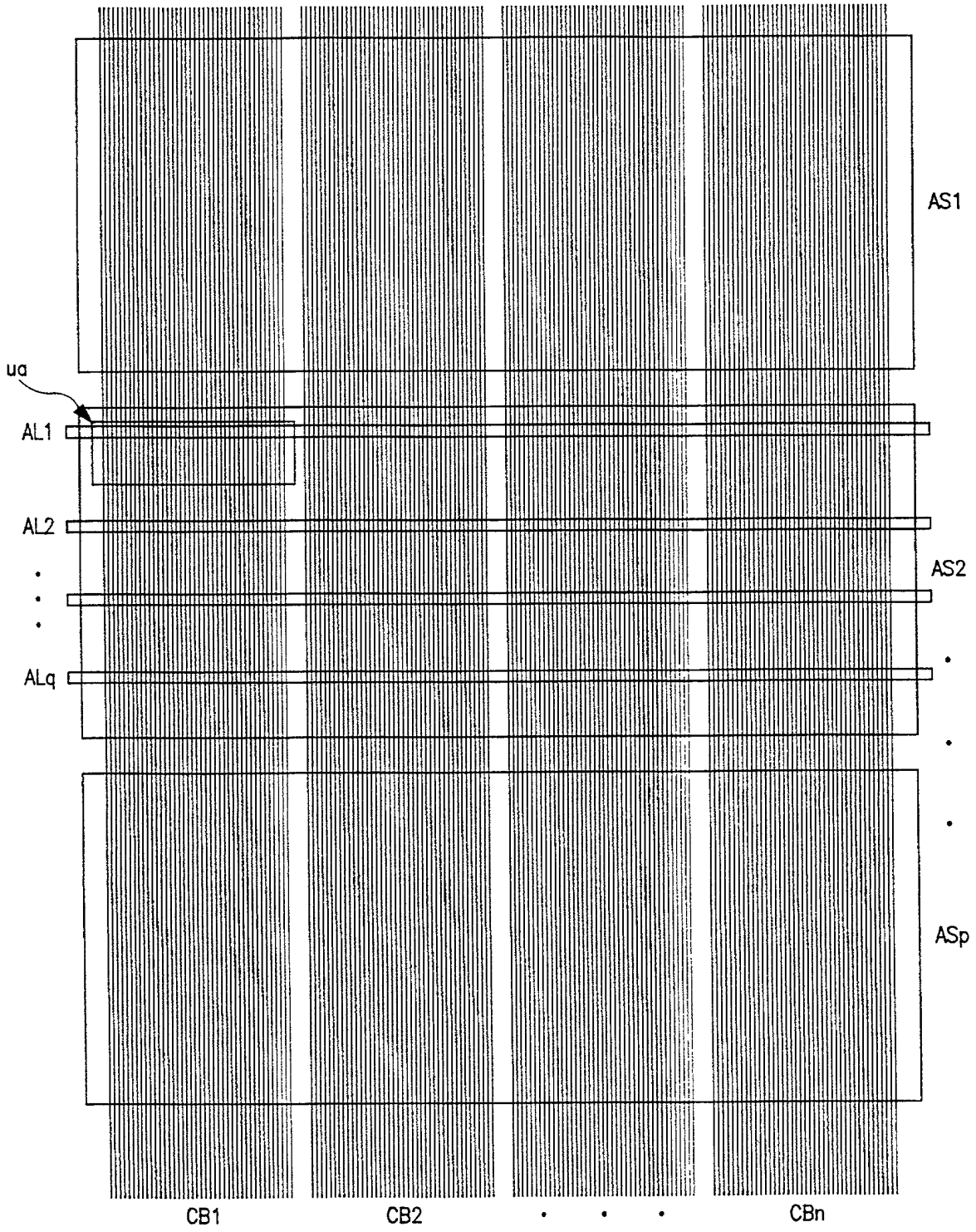


FIG. 2A

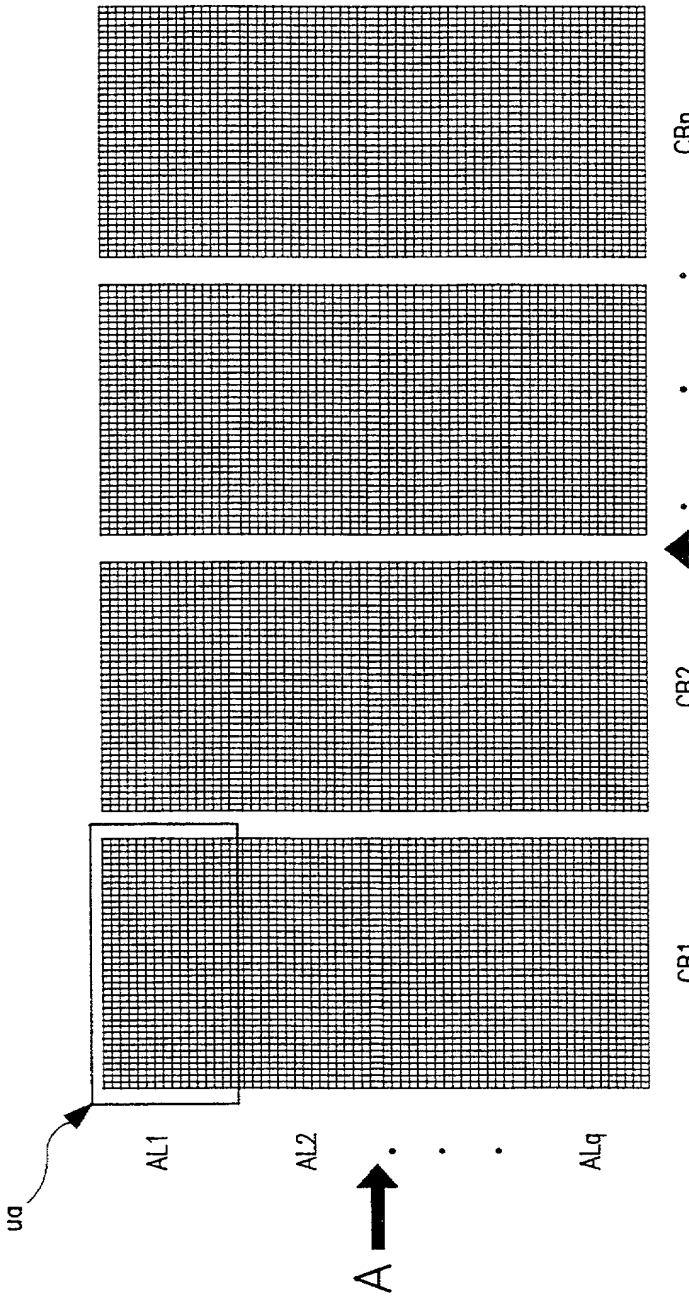


FIG. 2B

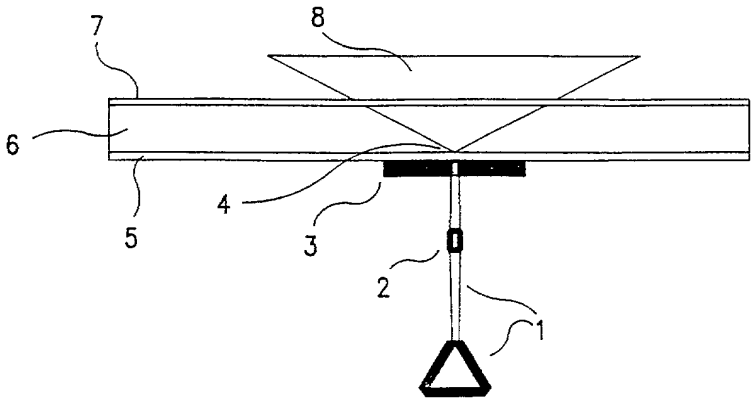


FIG. 3A

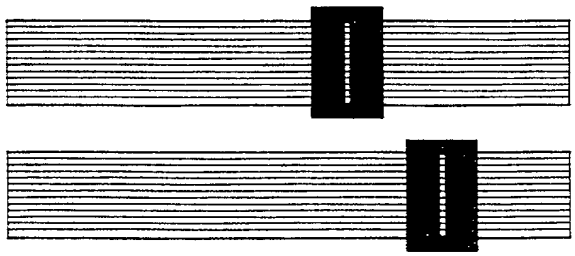


FIG. 3B

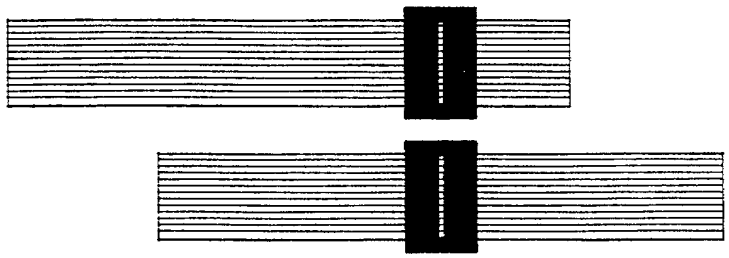


FIG. 3C

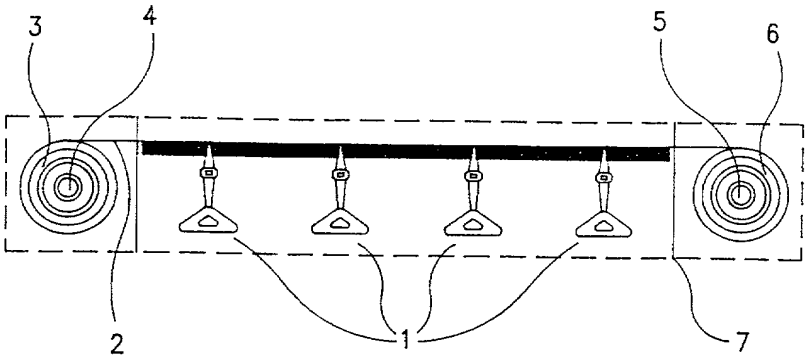


FIG. 4A

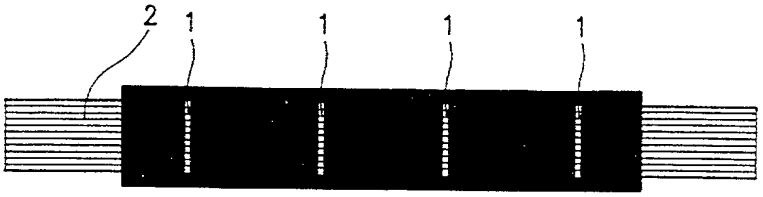


FIG. 4B

FIG. 5A

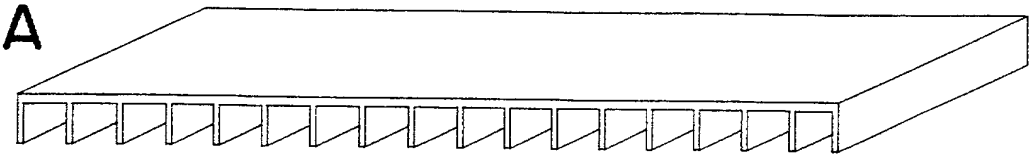


FIG. 5B

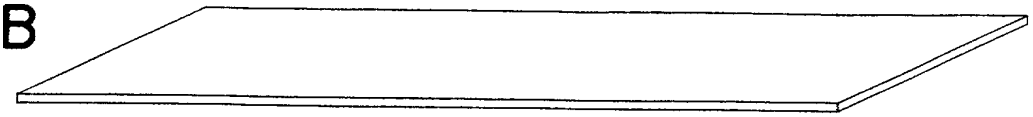


FIG. 5C

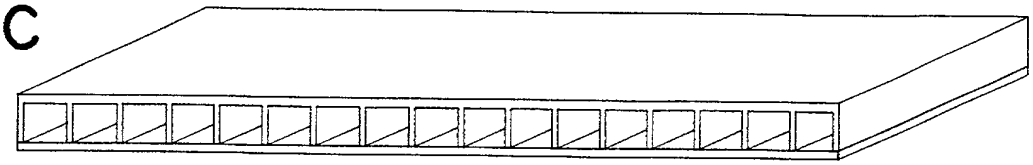
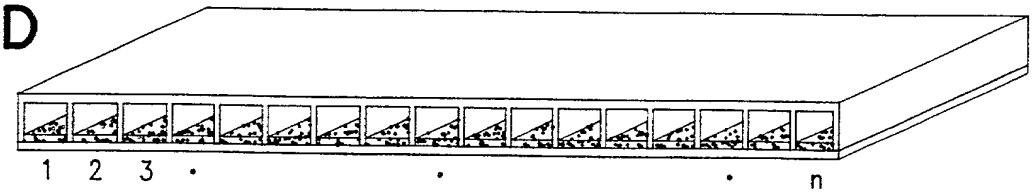
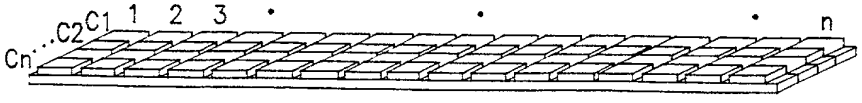


FIG. 5D



DDD

FIG. 5E



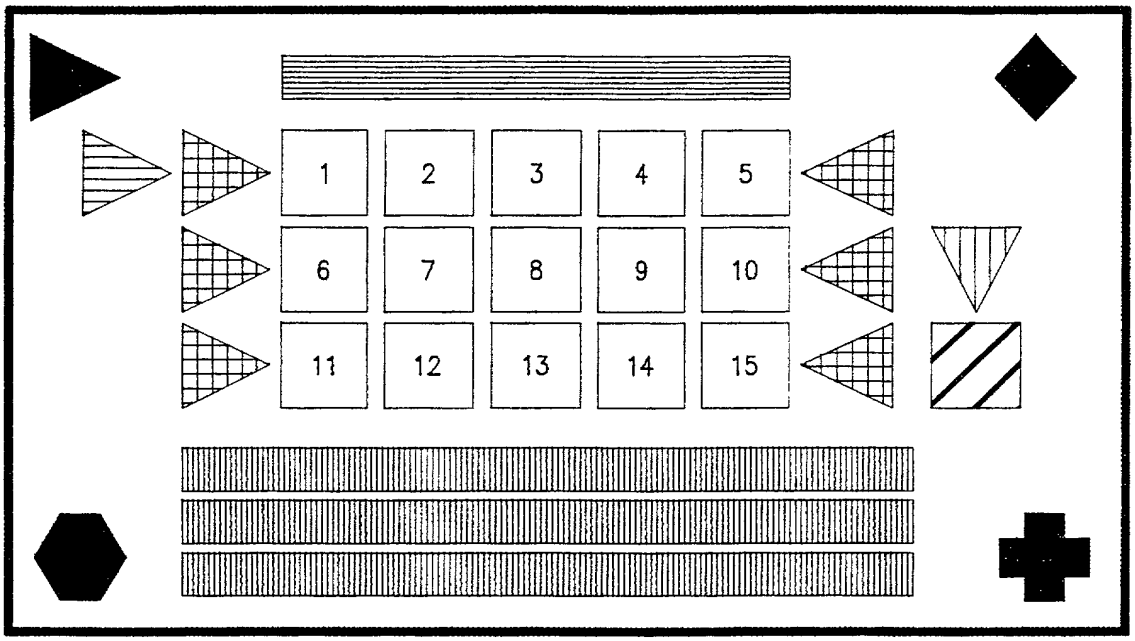


FIG. 6

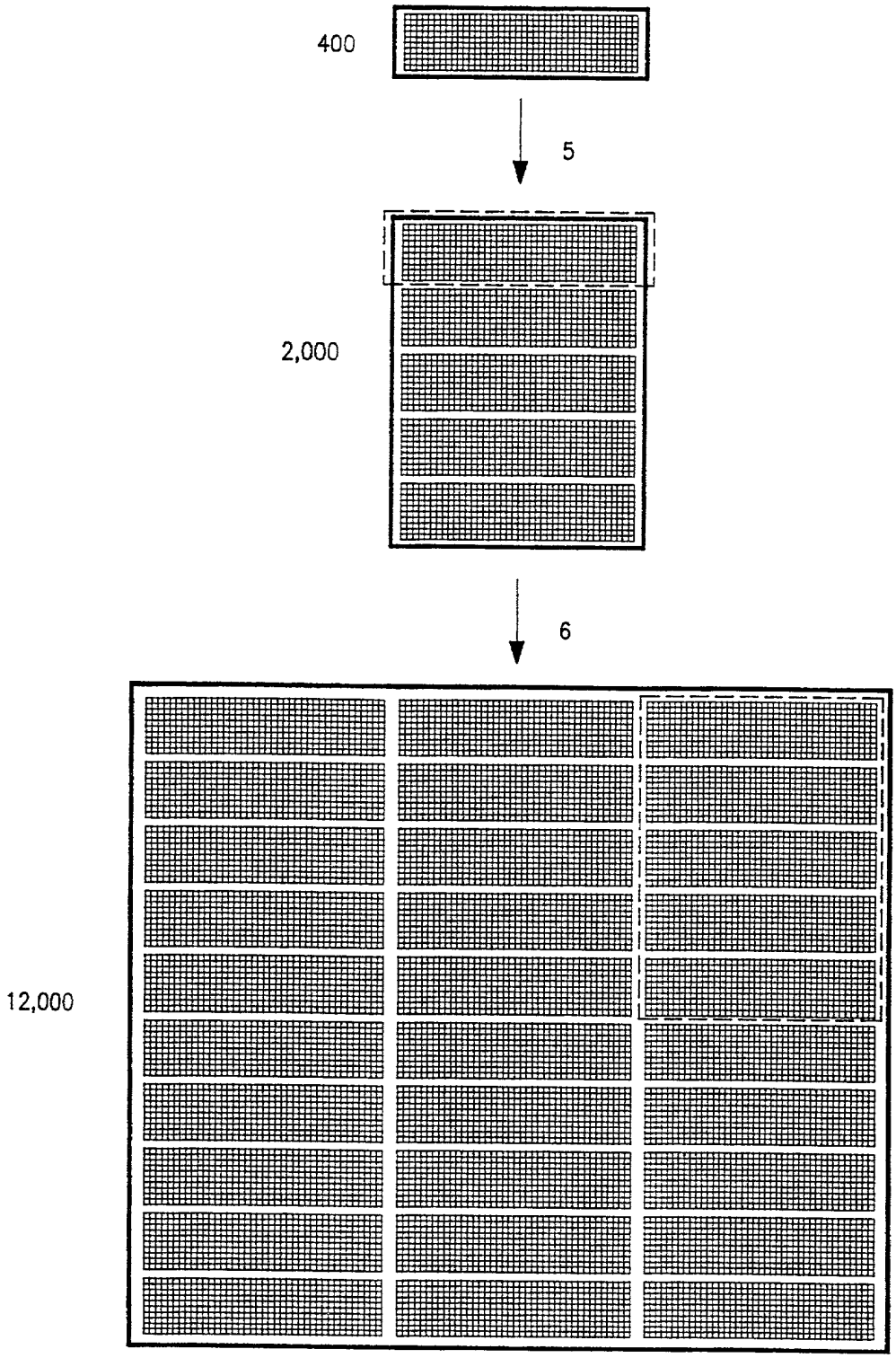


FIG. 7

FIG. 8A

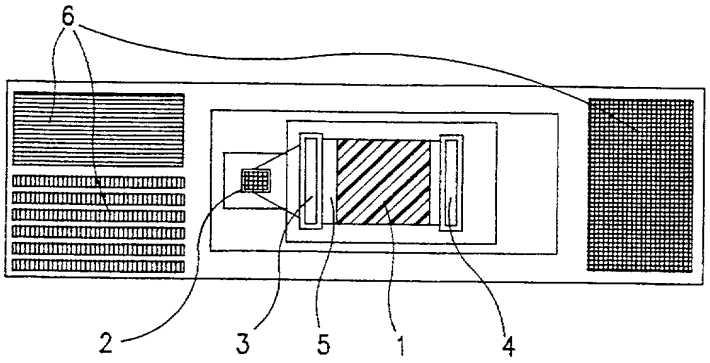


FIG. 8B

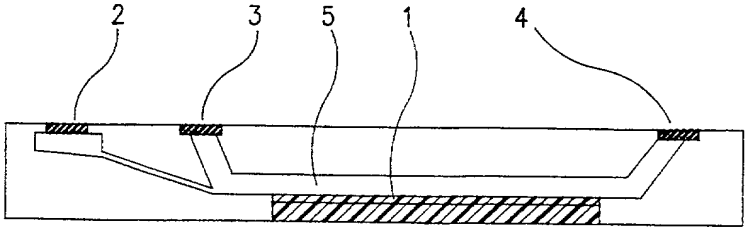
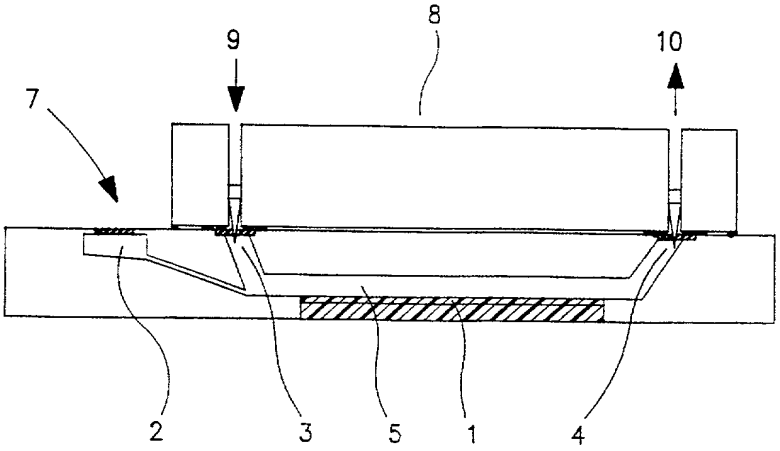


FIG. 8C



Attorney Docket Number: LAMII . 1

DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name,

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

METHOD OF MAKING PATTERNED ARRAYS OF ANALYTE-BINDING MOLECULES

the specification of which

- ☐ is attached hereto
- ■ was filed on 15 OCTOBER 1999 as United States Application Number or PCT International Application Number PCT/US99/23860 and (if applicable) was amended on _____

I hereby authorize our attorneys to insert the serial number assigned to this application.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN/PCT APPLICATION(S) AND ANY PRIORITY CLAIMS UNDER 35 USC §119			
APPLICATION NO.	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
PCT/US99/23860	US	15 OCTOBER 1999	YES
PCT/US98/21860	US	16 OCTOBER 1998	YES

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

PROVISIONAL APPLICATION(S) UNDER 35 U.S.C. §119(e)	
APPLICATION NUMBER	FILING DATE
60/104,642	16 OCTOBER 1998
60/104,643	16 OCTOBER 1998

I hereby claim the benefit under 35 U.S.C. §120 of any United States application, or §365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

PRIOR U.S./PCT INTERNATIONAL APPLICATION(S) DESIGNATED FOR BENEFIT UNDER 37 U.S.C. §120		
APPLICATION NO.	FILING DATE	STATUS — PATENTED, PENDING, ABANDONED

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected herewith: I. William Millen (19,544); John L. White (17,746); Anthony J. Zelano (27,969); Alan E.J. Branigan (20,565); John R. Moses (24,983); Harry B. Shubin (32,004); Brion P. Heaney (32,542); Richard J. Traverso (30,595); John A. Sopp (33,103); Richard M. Lebovitz (37,067); John H. Thomas (33,460); Catherine M. Joyce (40,668); Nancy J. Axelrod (44,014); James T. Moore (35,619); James E. Ruland (37,432); Jennifer J. Branigan (40,921) and Robert E. McCarthy (46,044); Larry S. Millstein (34,679);

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PATENT TRADEMARK OFFICE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of sole or first inventor (given name, family name) Larry S. MILLSTEIN	
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Residence	Citizenship
Post Office Address	

☐ Additional joint inventors are named on separately numbered sheets attached hereto.

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